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OVARIAN HORMONE THRESHOLD FOR EXPERIMENTAL MENSTRUATION IN MONKEYS

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The appearance of the first menstrual period, because it is such a clear-cut and obvious event, serves as the best criterion of sexual maturity in female primates. Menstruation may be induced experimentally, even in infant monkeys, by short treatment with ovarian follicular hormone. Determination of the amount of this hormone necessary to produce menstruation as immature animals approach sexual maturity should furnish a hormone threshold for adolescence of greater value than assays of urine for excreted hormone.

Other adolescent changes, such as development of the mammary glands, might be used as indicators, but these changes are gradual. The first ovulation, if it were possible to easily determine the time of this event, would probably be the final criterion of sexual maturity. In primates, however, ovulation occurs with so little outward change that until recently its time in the menstrual cycle has been much disputed.

In monkeys the first clear sign of approaching sexual maturity is the reddening of the "sexual skin," that of the region immediately surrounding the genital organs (1). Gradually the color deepens, the area affected spreads and the skin begins to swell. This reddening and swelling may fluctuate in degree for several months before the onset of the first menstrual period. Then follow the first menstrual cycles, the onset of menstruation usually being preceded by decrease in the intensity of the reddening of the "sexual skin" (2). These local cutaneous changes have been induced in ovariectomized monkeys by injections of ovarian follicular hormone (2), and therefore they are clearly indicative of increase in level of this hormone and offer an accurate criterion of effect of injected extracts.

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The following experiments attempt to find the amount of follicular hormone and the optimum time of treatment for the experimental production of menstruation in monkeys of different ages—immature, adolescent, and adult.

EXPERIMENTAL PROCEDURE. Seventeen female monkeys (Macacus rhesus) have been ovariectomized for this study. Of this group seven were mature, five adolescent, and five immature. Classification was made on a basis of 1, body weight; 2, presence or degree of reddening and swelling of the "sexual skin"; 3, size of the uterus, and 4, presence or absence of large follicles and corpora lutea at the time of ovariectomy.

After a post-operative recovery period, injections of water-soluble or oil-soluble hormone (theelin or amniotin)[2] were begun. Injections were given twice daily (8:30 a.m. and 4:00 p.m.) in an effort to maintain as nearly as possible uniform levels of hormone. The total dosages ranged between 78 and 2250 rat units, and the time of hormone treatment between 4 and 22 days.

The intensity of reddening of the "sexual skin" was followed as an indicator of the effectiveness of the hormone action. It appeared or became intensified after several days of injections. Following the cessation of injections the animals were observed closely for the onset of bleeding and records kept of the latent period; i.e., the time elapsing between the cessation of injections and the appearance of bleeding, and also of the duration of the flow. When it was certain that menstruation had ceased (or was not going to occur) injections were resumed. In all instances menstrual bleeding was observed externally. Bleeding so slight as to require microscopic identification of red blood cells in vaginal lavages was not recorded.

OBSERVATIONS. A total of 44 experimental menses was produced in seventeen ovariectomized monkeys of different ages; as many as five, and in one case eight, successive experimental menstrual periods in certain individual cases. In 15 instances the treatment failed to produce menstruation, either because of subthreshold doses of hormone or too short periods of injections. This made a total of 62 experiments.

I. *Mature ovariectomized monkeys.* These animals weighed from 4100 to 5700 grams at the time of ovariectomy. All had well developed "sexual skin" phenomena, had menstruated regularly, and had ovaries containing corpora lutea or large follicles.

In table 1 are listed experiments with oil soluble extracts of estrogenic material in ovariectomized adults. Animal 1 LE was given four series of injections ranging from 9 to 20 days in length. The first experiment began 31 days after ovariectomy and a total dose of 138 rat units of hormone

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was injected over a period of 20 days. After a latent period of 3 days she menstruated for 3 days. Four days after the first experimental menses had terminated, a second series of injections was given in which the total dose was reduced to 78 rat units during 20 days. Although producing some reddening of the "sexual skin," this treatment was not followed by menstrual bleeding during the following 50 days. This was probably a subthreshold dose over this interval of time. When in the third series of injections a dose only slightly larger (81 rat units) was given in less than half the time (9 days), it was followed by a latent interval of 6 days and then 2 days' menstruation. A fourth series in this animal, 230 rat units in 14 days, failed to produce menstruation.

TABLE 1

*Experimental menses in ovariectomized mature monkeys following injections of ovarian follicular hormone in oil solution**

MONKEY NUMBER	INJECTION SERIES	DAYS INJECTED	TOTAL DOSE (RAT UNITS)	LATENT PERIOD	DURATION OF MENSES
1 LE	1	20	138	3	3
	2	20	78		None
	3	9	81	6	2
	4	14	230		None
2 RE	1	20	78		None
	2	9	122	7	2
	3	14	117	4	3
	4	10.5	138	3	1
3 N	1	14	95	4	1
	2	14	190	5	6

* In these experiments doses were gradually increased to a maximum 4 or 5 days before cessation of injections.

The details of similar experiments in animals 2 RE and 3N may be followed by reference to table 1.

It will be noted that in two instances 78 rat units of oil-soluble extract in 20 days proved to be a subthreshold dose, while 81, 95, 117, 122 rat units (and larger doses), given over an interval of 9 to 14 days, proved adequate. In the above experiments extracts were dissolved in oil, which would favor slow absorption and also gradual fall in hormone level after cessation of injections.

In table 2 are listed experiments with a water soluble preparation of theelin, also in adult ovariectomized monkeys. For purposes of standardization of treatment, the daily dosage was maintained at the same level throughout the experiment. Injections were made twice daily.

Monkey 5 M is discussed briefly as a "sample" of this group. She had 5 successive experimental menstrual cycles. For the first three, the daily dose of 50 rat units was maintained, but the period of injections varied from 15 to 8 and 6 days, with correspondingly decreasing total doses. The post-injection period of 16 days before the onset of menses was the longest latent period, and the resulting menstrual period the shortest, observed in any of our experimental animals.

In monkey 5 M the first measurement of the uterus (24 x 18 mm.) was taken at the time of ovariectomy, which was done shortly after ovulation

TABLE 2

*Experimental menses in ovariectomized mature monkeys following injections of ovarian follicular hormone**

MONKEY	EXPERIMENT	DAYS BETWEEN LAST MENSES AND NEXT TREATMENT	HORMONE INJECTED (RAT UNITS)		DAYS INJECTED	LATENT PERIOD	DURATION OF MENSES
			Daily	Total			
4 M	1		100	2200	22	5	6
	2		100	400	4		None
	3	1	100	600	6	Died	
5 M	1	15	50	750	15	6	6
	2	1	50	400	8	6	5
	3	1	50	300	6	16	1
	4	1	15	150	10	11	2
	5	5	40	400	10	9	3
6 M	1	15	150	2250	15	6	7
	2	1	150	1350	9	8	6
	3	1	30	180	6	10	3
	4	2	15	150	10		None
	5	30	40	400	10	6	5
7 M	1	1	30	300	10	6	5

* In these experiments injections were maintained at a uniform level throughout.

had occurred (table 3). One hundred and thirty-seven days after ovariectomy and 2 days following cessation of the fifth series of injections the uterus was slightly smaller (22 x 18 mm.). A third measurement (25 x 20 mm.) made on the last day of treatment, 21 days after the second laparotomy, showed the uterus larger than before ovariectomy. The hormone treatment given was adequate, therefore, to prevent involution of the uterus over a relatively long period (158 days).

A large dose and a long treatment (5500 rat units in 15 days) was given ovariectomized adult 6 M for her first series of injections and this was followed by a second series much above the minimal level. Unusually long

menses, 7 and 6 days respectively, with profuse flow, followed latent periods of 6 and 8 days. Reduction of dosage and time of treatment in her third series of injections was followed by increase of latent period and decrease of duration of flow. Further reduced dosage in the fourth series failed to produce menstruation.

The uterus of animal 6 M showed very little change in size over a period of 162 days (table 3). In other words, a total dose of 4,330 rat units of theelin alone, given in 5 treatments totaling only 50 days of the 162 day period (a total of 112 days without hormone), was sufficient to maintain this adult uterus at its "ovulation time" level.

In summarizing results from this series of injections of water-soluble follicular hormone in ovariectomized adults, it is clear that 180 rat units

TABLE 3

Measurements of uteri

GROUP	MONKEY NUMBER	SIZE AT OVARIECTOMY	INTERVAL BETWEEN MEASUREMENTS	SIZE AT 2ND MEASUREMENT*	INTERVAL IN DAYS BEFORE NEXT MEASUREMENT	SIZE AT TERMINATION OF INJECTIONS
		mm.				
Immature	13 I	12 x 8	110	12 x 8		
	16 I	14 x 5	87	15 x 6		
	17 I	10 x 5	87	12 x 9		
Adolescent	8 A	20 x 17	146	20 x 16	22	20 x 17
Mature	4 M	26 x 20	90	20 x 9		
	5 M	24 x 18	137	22 x 18	21	25 x 20
	6 M	22.5 x 17	140	22 x 15	22	23 x 17

* At laparotomy in animals 5 M, 6 M, and 8 A; after the last injection in animals 4 M, 13 I, 16 I, and 17 I.

of hormone (in one case 150) equally distributed in 10 days, was above the hormone threshold for menstruation. Smaller doses of oil soluble hormone (81 r.u.) were effective. Also the hormone level sufficient to produce menstruation was adequate to maintain the uterus at its size at the time of ovariectomy and to prevent "castrate" atrophy of the mammary glands in adults, for the mammae of the injected monkeys were equivalent to those of fully developed normal, non-pregnant animals.

II. *Adolescent ovariectomized animals.* The 5 animals of this group showed reddening and swelling of the "sexual skin" but had not menstruated, and at the time of ovariectomy the size of the uterus and absence of corpora lutea or large follicles in the ovaries clearly indicated that they should be classed as approaching sexual maturity (table 4). The body weight ranged from 3300 to 4600 grams.

In animal 8 A the first 3 experimental periods were begun with a daily dose of 100 rat units and the periods of injections were 22, 3, and 6 days respectively. In the first series, the injection of 2200 rat units of hormone was followed by a latent period of 6 days and profuse menstruation lasting for 6 days. The day after cessation of this menstruation the same daily dosage was given for three days. Observations for 14 days thereafter failed to disclose any bleeding. It is probable that 3 days is too

TABLE 4

*Experimental menses in ovariectomized adolescent monkeys following injections of ovarian follicular hormone**

MONKEY	EXPERIMENT	DAYS BETWEEN LAST MENSES AND NEXT TREATMENT	HORMONE INJECTED (RAT UNITS)		DAYS INJECTED	LATENT PERIOD	DURATION OF MENSES
			Daily	Total			
8 A	1		100	2200	22	6	6
	2	1	100	300	3	(14)	None
	3	14	100	600	6	9	4
	4	6	40	400	10	10	2
	5	6	60	600	10	8	2
9 A	1		30	450	15	6	2
10 A	1		40	400	10	10	3
	2	2	25	250	10		None
	3	58*	30	300	10	(14)	None
	4	83*	40	400	10	8	1
	5	15	35	350	10	10	1
11 A	1		30	300	10	9	4
	2	1	30	300	10	7	3
	3	50	25	250	10	7	3
	4	7	20	200	10	6	2
	5	17	20	200	10	7	1
12 A	1		30	300	10	(15)	None
	2	15*	30	300	10	6	2

* Or after last injection if treatment failed to produce menstruation (see column 8).

short a time to induce sufficient growth of the endometrium. The same daily dose in the third series, 100 rat units for 6 days, produced a menstrual period of 4 days but the latent period was increased to 9 days. The uterus measured approximately the same at the time of the last injection, 168 days after ovariectomy, as at the time of the ovariectomy.

Monkeys 10 A and 11 A are of special interest, as 5 successive series of injections were made retaining a 10 day period of injections in each case.

After 400 rat units had produced a menstrual period of 3 days in monkey 10 A, a second series of 250 units failed to produce menstruation. In the third experiment begun 58 days later, 300 rat units also failed to produce menstruation. A fourth experiment with a total dose of 400 rat units resulted in menstruation for one day after an 8 day latent period. When the experimental menses lasted for a day only, the flow was usually slight. In all cases, however, it was readily apparent without need for identification of red blood cells by the lavage method. In adolescent animal 11 A, dosages of 200 to 300 rat units in 10 days were clearly above menstrual threshold. It will be noted that duration of menstrual flow decreased with decreasing doses of hormone.

From these experiments it was concluded that a 3 day period of injection is too short a time (monkey 8 A), that 6 days may be adequate, but that probably 10 days of injections are nearer optimal for growth of the endometrium which apparently must precede menstruation. As the dosage is decreased there is a tendency for the resulting menstrual periods to be shortened and the amount of flow to be decreased. As in the previous experiments in adult monkeys, measurements of uteri from several of these adolescent monkeys at the end of injections show this dosage adequate to maintain, or in some cases enlarge, the uterus, even though considerable periods without hormone were interspersed between treatments.

III. *Immature ovariectomized animals.* Five definitely immature monkeys were ovariectomized for a fourth series (table 5). The body weight of 1500 to 1950 grams clearly indicated that they were much too young to be approaching adolescence. There was no reddening or swelling of the "sexual skin" and at ovariectomy both uterus and ovaries were distinctly infantile.

Animal 13 I is noteworthy as she was given 8 successive series of injections, in most instances 10 days in length. Total doses ranged from 300 to 1000 rat units. In the first series 780 rat units in 13 days produced a menstrual period of 4 days after a latent interval of 7 days. When the dose was dropped to 600 rat units and the injection period reduced to 10 days, the latent interval was increased to 10 days but menses lasted for 5 days. In the third series treatment for 6 days was effective. Other details are easily followed by study of table 5. In the last experiment 300 rat units in 10 days failed to produce menstruation. It is probable that this is a subthreshold dose for such an immature monkey.

On the day following cessation of treatment in the eighth series the uterus was found to measure the same as at ovariectomy (12 x 8 mm.), which was done 110 days previously.

TABLE 5

Experimental menses in ovariectomized immature monkeys following injections of ovarian follicular hormone

MONKEY NUMBER	TREATMENT	DAYS BETWEEN LAST MENSES AND THEELIN TREATMENT*	HORMONE INJECTED (RAT UNITS)		NUMBER OF DAYS INJECTED	LATENT PERIOD	DURATION OF MENSES
			Daily dose	Total dose			
13 I	1		60	780	13	7	4
	2	1	60	600	10	10	5
	3	1	60	360	6	10	3
	4	1	35	350	10	9	2
	5	6	60	600	10	6	3
	6	153	100	1000	10	9	3
	7	2	35	350	10	6	2
	8		30	300	10	12	0
14 I	1		30	300	10	14	0
	2	14	30	300	10	5	2
	3	1	25	250	10	14	0
15 I	1		30	300	10	14	0
	2	14	30	300	10	5	2
	3	1	20	200	10	14	0
16 I	1		30	330	11		0
	2	17	30	300	10	9	4
	3	13	60	600	10	7	4
17 I	1		15	165	11		0
	2	17	15	150	10		0
	3	25	60	600	10	7	4

Monkeys 14 I and 15 I were tried for 2 experiments in each with the same hormone level and injection period. The first treatment failed to produce menstruation in each case, while the second series was effective. This is similar to the results obtained in monkey 12 A (table 4). It is probable, therefore, that in the first experiment after ovariectomy 300 rat units in 10 days is definitely below the menstrual threshold for animals of this age. A third series of 200 rat units in monkey 15 I and 250 units in 14 I failed to induce menstruation. It seems definite that these are subthreshold doses for immature animals as far as the production of menstruation is concerned.

In animal 16 I a first experiment with a dose of 330 rat units failed to produce menstruation. Seventeen days after the last injection, the second treatment with 300 rat units was effective. This result is similar to the second series in animals 14 I and 15 I. There may have been some cumulative effect from the first treatment.

In animal 17 I, 165 and 150 rat units were clearly subthreshold doses.

The uteri of monkeys 16 I and 17 I on the day following discontinuation of the third series of hormone injections and 87 days after ovariectomy, were larger than at the time of ovariectomy (table 3).

In summary for this group it can be said that the menstrual threshold of ovarian follicular hormone in immature monkeys (300 r.u. positive 3 times, negative 3 times) is considerably higher than that in adult and adolescent monkeys. It is remarkable, however, that the uterus in such an immature condition reacts by menstruation after such brief treatment with follicular hormone. Some growth of both uterus and mammary glands was apparent at the end of injection experiments, in spite of the intervals without hormone between periods of treatment.

Discussion. Examination of the ovaries of monkeys after the first few menstrual cycles indicates in most cases that ovulation has not occurred, thus establishing menstruation without ovulation and corpus luteum formation as a common occurrence in adolescent monkeys (3) (4) (5). The anovulatory menstrual cycle is also the usual thing during the summer months in adult monkeys. Menses in these anovulatory cycles cannot be distinguished externally either in duration or amount of flow from those which have been preceded by ovulation. The vascular reactions as observed in menstruating endometrium in ocular implants are the same in both ovulatory and anovulatory cycles (6). The only sure method of distinguishing is by histological recognition of small bits of endometrium, but it is necessary to curette to make this diagnosis.

Menstruation has been produced experimentally in normal adult monkeys by destroying large follicles (2) (7), by removing one or both ovaries (2), by removing recent corpora lutea (8), or by sectioning nervous pathways to the ovaries either in the spinal cord or peripherally (9) (10).

Menstruation has been produced from interval endometria in ovariectomized monkeys by injections of ovarian follicular hormone (2) (4) (11) (12) (13). These studies have shown that the principal action of the injected hormone is to induce growth in the endometrium and that a certain amount of this is necessary before menstruation can occur. After injections are stopped, a latent period of several days intervenes and then menstruation sets in. These experimental menses are indistinguishable externally from normal menses. Werner and Collier (1933) and others have reported similar experimental menstrual periods with the use of theelin alone in ovariectomized women.

When the follicular hormone is followed by short treatment with the corpus luteum hormone, the endometrium undergoes transformation equivalent to that of the premenstrual stage of ovulatory cycles and then menstruates when treatment is stopped. Recently Engle and Smith (14); Hisaw (15); Engle, Smith, and Selesnyak (16); and Corner (17) have described postponement of the expected menstruation either in normal

animals or in theelin treated animals by injections of corpus luteum hormone. Although the corpus luteum may have this endocrine function as regards menstruation in the ovulatory cycle, the evidence cited above for anovulatory menstrual cycles and for menses induced experimentally by theelin injections in ovariectomized primates remains fundamental.

Menstruation has been the subject of several recent reviews to which reference is made for further discussion (18) (19) (20).

While it may be desirable for purposes of standardization to inject the same daily dose over a period of days, it would probably approach much nearer conditions of normal secretion of follicular hormone by the ovaries to begin with moderate doses and increase the amount to a maximum as in the first series of injections (table 1). This method should simulate normal conditions of ovarian secretion as follicles approach maturity.

It seems clear that 3 or 4 days at the hormone levels tested is too short an interval to produce the necessary growth changes in the uterus to put it into condition where menstruation will begin when the hormone is withdrawn. A period of 6 days may be adequate, but a longer period of injections reduces the latent period before onset of menstruation and more nearly approaches the time relations of the normal menstrual cycle.

Sometimes a dosage and time interval which is not effective in the first experiment in immature and adolescent animals may prove effective in a second experiment on the same animals. It is possible that there is some cumulative effect or that an effect on other related endocrine glands, perhaps the anterior-pituitary, may be carried over to the next experimental cycle.

As there were intervals of complete absence of hormone between successive series of injections, these experiments did not reproduce the condition in a normal animal during several successive anovulatory cycles. It is possible in the normal animal that there is always some ovarian hormone present, but that the amount fluctuates. For this reason maintenance of continued growth of mammary glands and uterus is hardly to be expected. It is worth emphasis in this connection, however, that the size of the uterus is maintained by the hormone treatment described;—the measurements of the uteri taken at the termination of injections were in all cases equal to, or greater than, those made at the time of ovariectomy. Also this hormone treatment maintained the adult condition of the mammary glands in the older animals and induced considerable growth of mammary duct systems in the younger animals.

It is probably not desirable to attempt to calculate on the basis of body weights clinical dosages of ovarian hormone from these results in monkeys. It seems reasonable, however, that much smaller doses than re-

ported recently in the clinical literature would be effective as therapeutic measures clinically. From studies of atypical growths resulting from massive doses of estrogenic substances in both mice and monkeys it would seem preferable to hold therapeutic doses to as near the effective threshold as possible (21).

SUMMARY

Menstruation is one of the best criteria of adolescence in primates. This study reports the amount of ovarian follicular hormone (theelin, amniotin), and time of treatment, necessary to induce experimental menses in ovariectomized monkeys of various age groups.

In ovariectomized adults small doses of hormone "condition" the uterus (produce an interval endometrium) so that cessation of injections is followed by menstruation. Larger amounts of hormone are necessary to produce menstruation in adolescent and immature monkeys than in mature ones. Large doses of theelin, when concentrated in periods of time less than 6 days, are less effective. It is probable that the minimal time for the establishment of theelin effects approximates 6 days and the optimum 10 or more.

When theelin treatment is concentrated in shorter periods than 10 days, larger doses are required and longer latent periods transpire before menstruation sets in. The duration and amount of flow are likewise diminished by decreasing the dosage and shortening the period of injections.

A dosage which proves adequate for the menstrual threshold maintains the size of the uterus and induces growth in the mammary glands, even though periods without hormone are interspersed with periods of injections.

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THE SITE OF ACTION OF BOTULINUS TOXIN

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The toxin of botulinus on administration to animals produces after a definite incubation period a paralysis of skeletal musculature, usually resulting in death by paralysis of the respiration. The incubation period is prolonged, or the onset of symptoms delayed, by the administration of anesthetics (Bronfenbrenner and Weiss, 1921, 1922, 1924). This might suggest an involvement by toxin of the nervous system as one of the obvious sites of anesthetic action, in spite of good evidence in the literature of a curare-like action on the periphery. We have therefore reëxamined the action of this toxin with special reference to the central nervous system, as a preliminary to the study of the effect of anesthetic.

After a comprehensive study of the toxin's action on frogs, dogs, etc., Edmunds and Long (1923) and Edmunds and Keiper (1924) decided that the action was curare-like, in that it left the muscle still directly excitable, but inexcitable indirectly through its end-plate. Our own results confirm this. The present work involves the further test of botulinus toxin from two angles. First, a relatively pure and high-potency toxin permitted massive dosage with a minimum of the action assignable to impurities or contaminants. Second, the electrometric recording of nerve responses has reached a stage where a direct effect on the nervous system could be detected if it were produced. The definitive action of the toxin still appears to be curare-like, at the myoneural junction. There also is an effect of large doses on the heart and on the central nervous system, which presumably indicates that like most agents with a specific biological action, the specificity should be put in terms of threshold. As a potentially general biologically injurious agent, the point of greatest susceptibility, or of injury by least dosage, is the myoneural junction.

Preparation and dosage of toxin. The toxin used in the experiments was prepared by growing a culture of B. botulinus type A on a 1 per cent glucose broth poured over an equal volume of finely ground beef heart tissues. At the end of 4 days of growth under anaerobic conditions the

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liquid portion of the culture was poured off, filtered through the Berkefeld "N" candle, and preserved in sealed tubes under CO_2. The toxicity of this filtrate was determined by intraperitoneal injection of mice weighing 18 to 21 grams, and the amount which killed these mice in approximately 48 hours was assumed to be a minimal lethal dose.

Over a period of several months while the work was in progress, this toxin was retitrated periodically and the minimal lethal dose was found to vary from 0.000003 to 0.00001 cc. As a rule, 250 mouse lethal doses of toxin were needed to kill a 2000 gram rabbit in 24 hours by intravenous injection. The killing effect on rabbits, however, was much less regular than in mice, for reasons that will appear below.

Apparatus. The apparatus employed for maintaining respiration is diagrammed in figure 1. *A* is a tin can of 6-inch diameter, with a movable disc *B* operated by a rod *D* through the end to adjust for various sized rabbits. A ring *C* fits the other end, sealing to *A* by a gasket and held by three wing nuts, their bolts being hinged for quick tightening in an

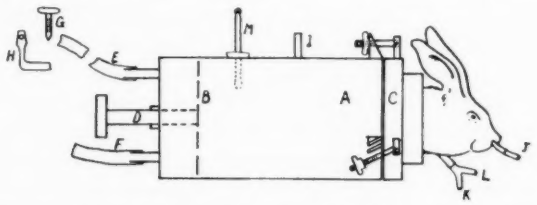

Fig. 1. Diagram of apparatus for artificial ventilation of paralyzed rabbits. Explanation in text.

emergency. This ring has tied over it the wrist of a rubber glove, the fingers cut off to leave a tube, through which the rabbit's head and one fore leg are pushed before applying *C* to *A*. *E* and *F* are rubber tubes connecting to two pairs of valves, one of each pair, *G*, being a needle valve to regulate rate of air flow, the other, *H*, a cut-off valve to regulate duration of opening. The latter valves *H* are operated by a motor driven eccentric, with such adjustments that the extreme position of opening, the length of stroke (and thence duration of open period) and the frequency of opening are separately regulated, and the two valves can be set to open at any phase relationships. A line from one valve runs to the vacuum supply, the other can be left open or carried to the pressure line. A side tube *I* can be used to record the pressure changes on a tambour, or when the respiratory machine is stopped, to record the animal's natural respiration. The insertion of the rabbit and the starting of the apparatus took about one-half minute, which in some cases proved too

long. The rabbit's fur was greased with vaseline, or adhesive tape applied, to seal the rubber sleeve. A thermometer was inserted at *M*.

Animals could be maintained in such an apparatus, after the failure of natural respiration, until accident intervened, with occasional administration of water or glucose solution. Usually the nostrils plugged with mucus, and glass tubes were inserted as at *J*, which required frequent cleaning. It was found more satisfactory, after the first few hours, or before an emergency arose, to insert a glass tracheal cannula, which could be done under ether without removing the animal from the respirator. A large opening at *K* permitted removal of mucus from the trachea with a pipe cleaner, and a side tube *L* served to record respiration by a tubulature to a bellows recorder, the aperture *K* being narrowed just sufficiently to give the desired sensitivity. During tests of natural respiration when the latter had nearly ceased (after apnea by artificial ventilation, or after partial paralysis), the complete closing of *K* allowed the pulse rate to be clearly recorded as a test of heart action.

EXPERIMENTAL. After the injection by ear vein of up to 3000 mouse M.L.D.'s of toxin per kilo, no perceptible symptoms are exhibited during the incubation period; after the first symptoms, of uneasiness and startled appearance, the train of events is the more rapid the larger the dose, but otherwise largely independent of dosage. Death in rabbits, without respiratory aid, is almost never due to failure of the primary respiratory musculature, but rather to inability on the part of the animal to open its external nares reflexly for inspiration. The rabbit, at least when intoxicated with botulinus toxin, cannot breathe through its mouth, even when the tongue is drawn forward and the jaws propped open. The external nares are closed passively by cartilaginous flaps, presumably to exclude dust and dirt during burrowing. The action of the toxin, involving difficulty in expelling mucus accumulations from the nasal passages, results in the accumulation of a partially dried viscous glue, which at an early stage of the weakening of the nasal musculature effectually seals the nostrils and the animal very promptly suffocates.

If asphyxia develops slowly, the stage of increased stimulation by oxygen lack is passed over, and depression results immediately. At this stage, a short period of artificial ventilation may revive a completely prostrate animal to the extent that for 15 minutes or less he breathes with sufficient effectiveness to appear almost normal; that is, increased oxygen restores the ability of the respiratory centers to function after the direct impairment of the peripheral mechanism has indirectly depressed the centers. Finally, after all respiratory movements have ceased, the animal can be kept alive by artificial ventilation, until some accidental interference with the artificially manipulated oxygen supply causes death.

At various stages after the failure of natural respiration to maintain adequate supply of oxygen, the responses of the animals have been tested, under ether anesthesia when necessary, and the results compared with the responses of normal animals under the same external conditions.

1. Failure of respiration. The respiration, normally rapid and shallow, slows before other signs of respiratory embarrassment are obvious, due to reduction of intake volume rather than to toxic effect on the respiratory center. With progressive slowing, the respiratory efforts become *weaker*, due to paralysis, rather than becoming stronger as they would in normal animals due to a corresponding degree of asphyxia. Finally the animal, now prostrate, ceases to breathe at all, ending with slow gasps accompanied by opening of the mouth and slight movements of the nose without significant movement of air. Terminal asphyxia results suddenly when the tidal air fails to ventilate the alveoli, and asphyxial depression of the center is added to toxic paralysis.

If just before death the animal is resuscitated, a fair degree of recovery of movements occurs. In other words, the central mechanism has been depressed by asphyxia rather than by the toxin. This course of events suggested the experimental procedure of recording at half-hour intervals the respiratory force and rate, and heart beat, of the intact animal, after artificial ventilation just sufficient to produce apnea, allowing the animal to proceed from this condition through successive stages of asphyxia to complete central paralysis, whereupon artificial ventilation was again applied. After even massive doses have resulted in an initial prostration that certainly, if not interfered with, would have caused immediate death, slight respiratory movements can be recorded after ventilation for a period of from six to twelve hours. After smaller but still fatal doses, respiratory movements can be detected in certain animals for at least two days. Often after no movement of air can be recorded even directly from the tracheal cannula at such a sensitivity that the heart beat is recorded via the lung passages with a stroke of the recording lever 3 mm. high, slight movements of the nose such as had previously been associated with inspiratory effort can still be observed. Just before this stage, and after long periods of adequate ventilation, it is found in some animals that the respiratory *center* (as indicated by the frequency and total number of respiratory efforts between apnea and asphyxia) is still behaving nearly like the normal under comparable conditions, although the peripheral effect of its activity on the musculature is almost zero.

In other cases, however, but only after heavy dosage, the center is probably affected. In these cases, after apnea, the respiratory efforts are abnormally infrequent, may approach the apneustic in type, and cease after a shorter period of asphyxia than previously in the same animal. In two cases we have observed further a complete dissociation

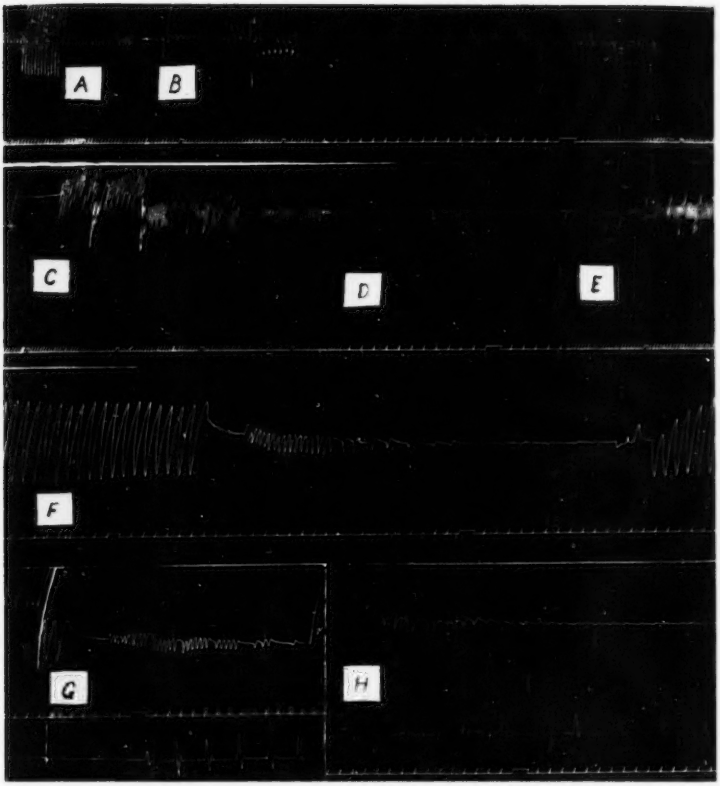

Fig. 2. *A*, artificial ventilation recorded from tracheat cannula, about 1 hour after first prostration of rabbit from respiratory paralysis, 100 M.L.D. intravenous. Two sizes of side-opening of cannula employed. *B*, ventilation stopped, side tube adjusted, record of natural respiratory movements. Large flings of lever are due to jerks of the animal's body. The pulse can be seen on the respiratory record in the fast period of the record. *C*, two hours after *B*, irregular natural respiration after apnea. The two large deflections are body movements. The regular strokes are normal respirations, with gasping or apneustic type movements interspersed, the latter associated with nose movements. *D*, faster kymograph speed, mostly gasping respirations persisting. *E*, artificial respiration restored, after opening side tube; gasping respirations superposed on this, with increased force as ventilation proceeds.

F, three hours after *B*, ventilation stopped, no respiratory movements except gasping motions of nose. The pulse record appears as the side tube of the tracheal cannula is closed. Probably heart block occurs rather than slowing, as seen in *G*, a similar record taken after 5 minutes more of thorough ventilation. On this record a tambour was added (bottom) for manual signaling of nose movements. These appear to be reflexly associated with the heart blocks, preceding the latter by about 3 heart beats at first, then by 2. *H*, after thorough ventilation for 15 minutes more, the heart has recovered from block, and now slows gradually, but at a much earlier stage of asphyxia than normal. Movements of nose associated with gasping respiration in the normal animal start at about the stage that the normal respiration should start after apnea. The heart fails before the respiration. Middle trace, signal of nose movements. Time in seconds for all records.

between two respiratory phenomena not dissociated in the normal. While the last traces of respiratory movement were still being recorded, a slower apneustic or gasping movement was added to it (fig. 2, C), observable particularly in the movement of the nose, and the two sets of movements were not in phase. Even after air movements could no longer be recorded, nose movements could be observed. These were suppressed during artificial ventilation, and reappeared at an early stage of asphyxia, not with the periodicity of normal respiratory movements but with the frequency typical of terminal gasping (fig.2, G and H). Since, as indicated by heart rate (see below), the animal was really not yet in the

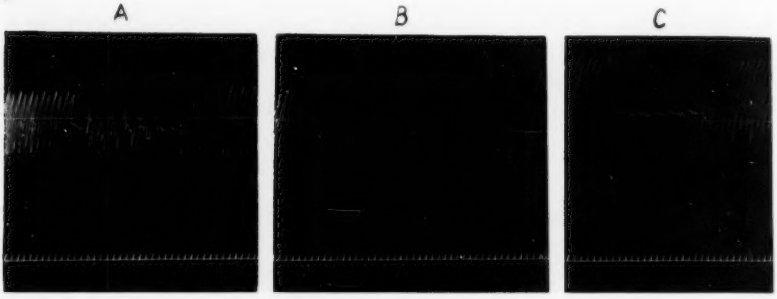

Fig. 3. Record from tracheal cannula of rabbit prostrated 48 hours earlier by partial respiratory paralysis, 20 M.L.D. intravenous. Respiratory movements ceased after 24 hours, but previous to this could be maintained for over a minute after apnea at a time when too little air was moved to ventilate the lungs. Time in seconds. *A*, artificial ventilation stopped, tracheal side-tube closed, pulse record from lungs showing 3–1 block immediately after ventilation. *B*, a similar record, allowed to run until only an occasional heart-beat was recorded. The apparent slowing of the heart is really heart block, for a very slight pulse can be read on the record between the slow beats, with the original rate. *C*, an attempt was made to release the block by over-ventilation, which reduced the force of the beat but only decreased the block ratio to 6–1.

stage of extreme oxygen want, it is believed that this type of respiration suggests direct effect of the toxin on the respiratory center, as expressed in both depression and dissociation.

2. Failure of the heart. In similar experiments the heart rate could be followed during the period between apnea and asphyxia. Some of these animals have died suddenly under direct observation with every evidence of adequate artificial ventilation. Realizing that the rabbit heart is rather liable to failure under experimental conditions, some of these deaths we still believe we can assign to heart impairment induced directly by the toxin.

The normal rabbit heart, under ether, with the chest opened, following

periods of artificial ventilation, may beat in the ensuing asphyxial period for as much as 2½ minutes after respiratory efforts have ceased. Our experimental animals, completely paralyzed by not too large doses of toxin, sometimes nearly duplicate this performance. Usually, however, this is not the case; the heart slows sooner than the normal during asphyxia after apnea, and may almost stop even before respiratory efforts have ceased (fig. 2, F, G, H). The only two animals which have died during actual observation of the heart record have died during asphyxial periods, not by sudden cessation of the normal frequency, but due to the persistence of the operator in recording the one more final beat which failed to eventuate, before turning on the air. In both cases, however, earlier in the experiment the heart had not slowed so much, nor—obviously—failed to recover at the same period after ventilation had stopped.

We feel that, while no one experiment is conclusive, the accumulated evidence suggests a direct effect of very large doses of toxin on the heart, but no appreciable effect of doses just sufficient to cause respiratory paralysis; that is, the heart is less sensitive to the toxin than is the skeletal motor apparatus. Stimulation of the vagus peripherally in a few experiments produced a fair slowing of the heart even in advanced stages of intoxication.

3. Nerve-muscle failure. After cessation of respiratory movements, and when artificial ventilation will no longer restore them by oxygenating the blood supply, stimulation of the peripheral stump of the cut phrenic nerve also fails to cause contraction of the diaphragm. Stimulation of the sciatic of a paralyzed animal also has no effect on muscles innervated. Direct stimulation of the muscles causes contraction, and the contraction is conducted along the fibers of a parallel fibered muscle such as the sartorius. We have not investigated to what degree such a contraction is of normal strength or time relations, but the facts are sufficient to identify a curare-like block at the myoneural junction, provided the nerves involved are also functional. Excised nerves from such animals give normal action potentials as recorded on the oscillograph. We thus substantiate the conclusion of Edmunds *et al.* that the myoneural junction is the critical structure whose failure causes death in botulism.

These experiments do not indicate whether the partial recovery from paralysis of asphyxiated animals due to artificial ventilation is a recovery of the myoneural junction or a recovery of the centers. The change in *frequency* of respiration on readministering air in the previous type of experiment is only conclusive in showing that the intoxicated center reacts qualitatively at least as the normal does. Since the myoneural junction of the normal warm-blooded animal is also affected by asphyxia, this recovery in the intoxicated animal may be viewed as the normal process of recovery acting in an impaired structure.

4. The phrenic neurogram. Animals which had received large doses of toxin, after paralysis of respiration, were given artificial respiration by tracheal cannula under ether, the chest opened, the phrenic nerve cut next to the diaphragm and dissected free up to the level of the base of the heart. The end was crushed and mounted on the amplifier grid electrode, a ground electrode was attached 1 cm. or more away, and a second

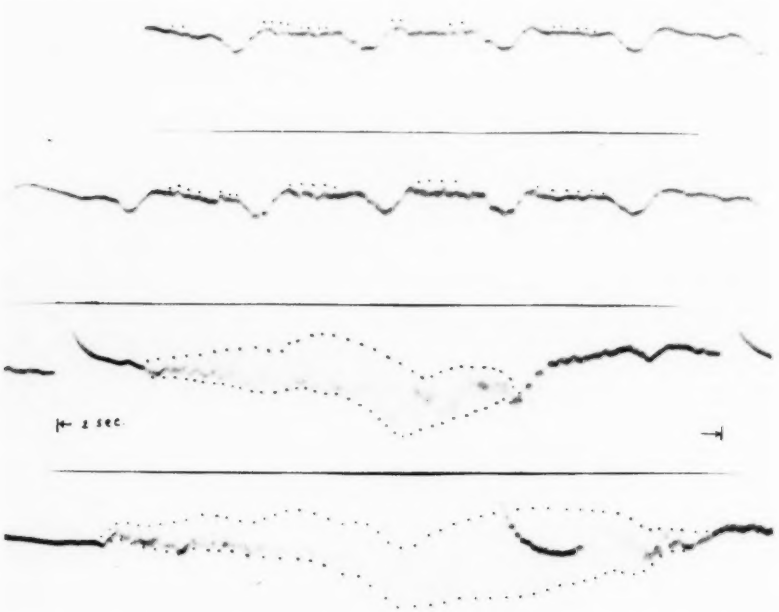

Fig. 4. Phrenic neurograms of normal rabbit, 1st, 3rd, 16th and 31st inspirations after apnea. In the first two records the heart beat is recorded due to mechanical jerking of the nerve on the electrodes. Heart rate 4 per second. In the third strip the time between two upward breaks in base line is 1 second. The last record shows the maximum amplitude of the series, but duration of discharge was still more prolonged in subsequent inspirations. Dotted lines trace the faint outlines of the amplitude.

ground made contact with the nerve near its attachment. Ventilation was applied until the phrenic neurogram as observed on the oscillograph was absent, that is, until apnea was induced. A camera was then started which drew a 60 mm. wide strip of bromide recording paper across the face of a second oscillograph tube, operating in parallel to the observation tube from the same amplifier. After ventilation was stopped, sample respirations were recorded until either the heart slowed dangerously or the

respiratory responses grew brief and weak. Normal animals were similarly recorded as controls. The lungs were collapsed throughout the periods between ventilations.

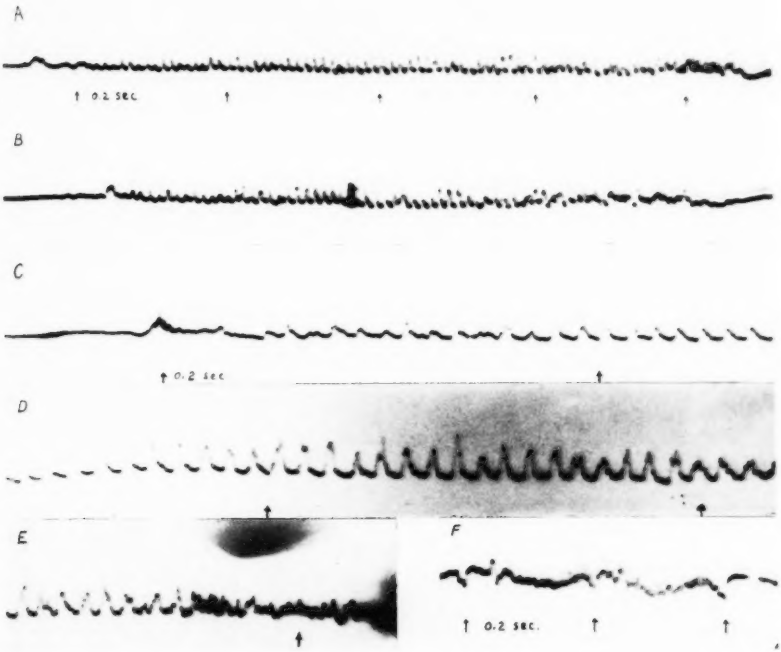

Fig. 5. Phrenic neurograms of intoxicated rabbit after respiratory paralysis, 20 M.L.D. intravenous. First strip, 8th inspiration, second 15th after apnea, increase in amplitude but not in duration. Arrows mark $\frac{1}{5}$ second, recorded by short shocks too faint to reproduce. Strips 3, 4, and first half of 5, the 10th inspiration with faster movement of paper to show striking synchronism of volleys, which, however, is probably not assignable to the toxin. Time in $\frac{1}{5}$ second, records continuous one with the next. Note breaking of synchronization at end of inspiration. Last record, 15th discharge from a different rabbit, lasting $\frac{1}{5}$ second, maximum duration of which this animal was capable, 20 M.L.D. intravenous. Heart beats recorded due to mechanical movement of nerve on electrodes, 4 per second. Shortening of duration of discharge and earlier failure under asphyxia presumably assignable to toxic action on center.

In the normal animal under light anesthetic, thirty to fifty respirations can be recorded between apnea and asphyxia, a period lasting up to two minutes. Each discharge down the phrenic consists of a series of volleys more or less discrete (Gasser, 1928), starting and ending at low amplitude

but of approximately constant frequency when distinct enough to measure. Successive discharges may increase in duration and amplitude up to at least the thirtieth (fig. 4), then decrease in amplitude while still increasing in duration, and finally decrease in both amplitude and duration to cessation. The heart may or may not have slowed before respiration ceases, but may continue to beat for at least 2 minutes longer.

In some animals, after complete paralysis of the respiratory musculature, the phrenic discharge is normal, and the changes in its character during asphyxia follow the normal course (fig. 5, A–E). In others, in which the heart action has been still good, the duration of the separate respiratory discharges has been distinctly briefer than normal, and the time of cessation during asphyxia also less (fig. 5, F). The appearance is that of a center more easily fatigued, and therefore less able to withstand withdrawal of oxygen. This is a well-known effect of curare on the myoneural junction, and presumably connotes here a depression of central nerve synapses, but only, it is obvious, at concentrations much above the threshold for muscle. It happens that our most clearly reproducible records are those from intoxicated animals, and these are clear because of the close synchronization of the discharges in separate fibers taking part in the volley (fig. 5, A to E). This synchronization cannot be taken as an effect of the toxin, however, since the phenomenon is observed also in the normal animal (Gasser) and these particular animals happen not to have shown any undue signs of depression assignable to the toxin; their discharges approached the normal in duration and number. It can be concluded that depression in peripheral nerve is not involved in any toxic action exhibited by the intact animal, and that the gradual and progressive depression of respiratory function indicated in the kymograph records of the longer lasting experiments on intact animals involves in part a central depression such as is found here.

5. Peripheral nerve. In treating excised tissues with toxin, even more than in treating the animal as a whole, the effects of substances other than toxin in the material containing it become important. For instance, nerve is notoriously responsive to changes in certain salt concentrations, and it could be anticipated that to obtain any effect the dosage of toxin would have to be extreme. Since the toxin can be inactivated by heating, control nerves were exposed to heated toxin. The straight solution heated or not had a depressant action. In toxin diluted with equal parts of Tyrode solution the nerves were gradually depressed, but recovered promptly in Tyrode to a state not far different from that when kept in Tyrode alone. A pair of vagus nerves from an animal receiving 40 lethal doses were removed after complete paralysis of the animal and kept for $5\frac{1}{2}$ hours in the Tyrode-toxin mixture containing 10,000 mouse M.L.D.'s

per cc., at 6°C. They were tested initially, after 3½ hours, and after 5½ hours, one each first in the solutions of active and inactive toxin and then after a brief soaking in Tyrode. In the toxin solutions they were depressed, but not to extinction of action currents. In Tyrode they returned promptly to a reasonable approximation of their original activity, the one from the active toxin being in as responsive a state as the one from the inactivated solution. This and other modifications of the procedure showed no effect whatever attributable to the toxin in peripheral nerve.

CONCLUSIONS

Rabbits intoxicated with varying dosages of botulinus toxin have been maintained in an artificial respiratory apparatus after complete paralysis, and their condition tested. Death without artificial interference ensues not when paralysis is complete but when the weakening of some critical musculature prevents the tidal air from ventilating the alveoli. At this stage of paralysis, if oxygen is supplied artificially, skeletal muscular activity is by no means abolished.

The immediate cause of death in rabbits is usually the sealing of the external nares by dried mucus, at a stage when the nasal musculature is just weakened sufficiently to prevent reflex opening. This circumstance may account in part, but not wholly, for the variability of the M.L.D. for rabbits.

At the time of first prostration, which, if aid is not administered, is followed very promptly by suffocation, animals can still respire sufficiently to maintain life if the air passages are kept open; and if artificial ventilation is supplied, respiratory movements may be observed for many hours after prostration, the time depending on the dosage.

Death without artificial ventilation always occurs from paralysis of the myoneural junction by a curare-like action of the toxin, or by heart failure, probably heart block, of which partial asphyxia is the immediate cause. With massive doses, and long after paralysis of skeletal musculature, partial or complete heart block and depression of the respiratory center under artificial ventilation can probably be assigned to direct toxic action on these structures. Under the action of toxin in large doses, such effects are induced by a degree of asphyxia decidedly less than that required in the normal animal, or occur in spite of normally adequate ventilation.

The specificity of the toxin for the myoneural junction is at least as great as that of other pharmacological agents for their characteristic sites of action.

No effect of toxin in any concentration that could be employed, in the body or out, could be observed on peripheral nerve.

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THE TETANY OF OESTRUS IN THE PARATHYROIDECTOMIZED DOG

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It has been known for some time that some women after thyroidectomy suffer attacks of tetany at each menstrual period, but it remained for Luckhardt and Blumenstock (1922) to call attention to the rôle of the parathyroids in these phenomena. These workers showed that female dogs parathyroidectomized some months before oestrus, treated with intravenous Ringer's solution immediately after the operation to control the tetany, again showed symptoms characteristic of parathyroid deficiency at the onset of the next oestrous cycle (anorexia, hyperpnea, retching, etc.). Luckhardt and Blumenstock were unable to explain the recurrence of severe tetany during the oestrous cycle, months after the operation, but suggested that the symptoms pointed to a general paresis of the sympathetic system. The earlier observations were confirmed by Dragstedt, Phillips, and Sudan (1925), who likewise were unable to explain the cause of this tetany.

While we were making observations on the blood chemistry of parathyroidectomized dogs in the latent survival period, an opportunity was afforded to determine several inorganic ions before and during oestrus in the parathyroidectomized dogs in our series of experiments. We hoped to throw some light on the problem by determining whether or not oestrus caused a further lowering of serum calcium which might explain the tetany in oestrus of the parathyroidectomized dog.

EXPERIMENTAL. Healthy normal female dogs were selected from the animal colony and after an adequate control period, a bilateral thyroparathyroidectomy was performed under ether-morphine anesthesia. The thyroids were carefully removed and the thyroid vessels ligated as close to the carotid artery as possible. In all the dogs the characteristic symptom complex of parathyroid deficiency developed within 48 to 60 hours after the operation if the calcium was removed from the diet.

Calcium lactate (Luckhardt and Goldberg, 1923), and calcium gluconate (Szurek, 1929) were given in doses of 1.5 and 3.0 grams per kilo, respectively, each day in the food or by stomach tube. This procedure was carried out for 30 to 60 days and by this means postoperative tetany

was controlled. The diet was bread and meat, care being taken not to give too much of the latter.

When all symptoms of tetany had disappeared, these dogs were considered to be in the "survival" period. Blood samples were taken at convenient intervals and the animals carefully observed for any signs of tetany. If any signs of tetany developed they were relieved by calcium therapy. On no occasion was a blood sample taken until 12 to 18 hours had elapsed following the dog's last meal.

Calcium, phosphorus, sodium, potassium and chlorides were determined in duplicate on the sera of these animals. The methods used were the following:

Chlorides—Wilson and Ball (1922)
Calcium—Kramer and Tisdall (1921)
Phosphorus—Fiske and Subbarow (1925)
Sodium—Kramer and Gittleman (1924)
Potassium—Kramer and Tisdall (1921)

Blood chemistry studies were also made on three normal dogs on a similar diet for some time before, during, and after oestrus. In these animals, calcium, phosphorus, pH and CO_2 were determined on the serum and data of one of these dogs as shown in table 1 to serve as a comparison with those data obtained in the parathyroidectomized series.

RESULTS AND DISCUSSION. In table 1 are given data on four normal female dogs and, for sake of space economy, the condensed protocols are shown for only three of our six parathyroidectomized female dogs that came into oestrus during the latent tetany period.

These data illustrate that in the normal dog there is no significant change in the values for blood calcium, phosphorus, pH and CO_2 during anoestrum, proestrum, oestrus, or metoestrum. The values for the inorganic ions and pH and CO_2 are quite within the normal range.

The data shown for dog 6 are characteristic of our findings for the parathyroidectomized female dog in oestrus in the latent tetany period. During anoestrum, her blood picture shows a remarkably low serum calcium. There was no marked change in proestrum. On the second day of oestrus the blood picture was practically the same, but mild tetany was evident. No change in her behaviour was noted on the seventh day of metoestrum, but the serum calcium was found to be slightly raised. This parathyroidectomized dog, then, illustrates a case of low serum calcium during oestrus, with a rise in metoestrum, and only a very mild tetany.

Dog 5, on the other hand, suffered severe attacks of tetany in oestrus, although her blood values were close to the normal. On the third day of oestrus, attempts were made to service the dog after a blood sample

TABLE 1

DATE	Ca	P	Na	K	Cl	pH (20°C)	CO_2	REMARKS
					(a) Normal			
								Dog 1
7-24-29	10.6	3.9	326	22.9	627			Definitely in oestrus
7-31-29	10.8	3.8	339	23.1	649			Metoestrum. Will not accept male
								Dog 2. Accepts male
7-18-29	12.8	4.8	326	25.3				Oestrus
7-19-29	12.6	4.6	328	25.3	668			1st day metoestrum
								Dog 3
1-10-33	11.44	5.7				7.56	52.7	Anoestrus
1-26-33	12.20	5.3				7.51	58.4	2nd day proestrum
1-31-33	12.88	4.6				7.53	57.2	1st day oestrus
2- 5-33	12.63	4.5				7.56	55.6	6th day oestrus
2-12-33	12.60	4.5				7.64	57.2	Metoestrus
								Dog 4
3- 5-33	11.70	3.6				7.54	56.2	Anoestrum
3-11-33	11.62	3.7				7.56	55.2	3rd day proestrum
3-15-33	11.84	3.8				7.59	57.9	1st day oestrus
3-19-33	11.78	3.6				7.55	54.1	5th day oestrus
3-28-33	11.54	3.8				7.57	55.8	Metoestrum
					(b) Parathyroidectomized			
								Dog 5 Parathyroidectomy operation 10-27-28. Last tetany 11-26-28
12-18-28	7.8	4.5						No tetany
1-21-29	7.4	5.7	414	22.0				No tetany
5-13-29	8.7	6.3	322	24.0	661			No tetany
5-27-29	9.8	6.0	348	21.6	650			Dog definitely in oestrus. Coitus and tetany
6- 5-29	7.4	6.5	380	20.3	649			No tetany. Metoestrum
6-25-29	11.3	5.6	391	21.9	668			No tetany. Dog pregnant
7-26-29	6.5	6.4	315	22.9	628			No tetany
8-25-29								Dog aborted 13 cm. dead fetus. No tetany
								Dog 6 Parathyroidectomy operation 4-29-29
5-16-29	5.9	6.3			668			No tetany
5-21-29	5.5	5.8	315	24.5	694			No tetany
5-23-29	5.1	4.6	344	22.1	682			No tetany. Proestrum

TABLE 1—*Concluded*

DATE	Ca	P	Na	K	Cl	pH (20°C)	CO_2	REMARKS
(b) Parathyroidectomized—*Concluded*								
5–27–29	4.8	4.0	298	20.4	658			Definitely in oestrus Few tremors, but no marked signs of tetany Gave no medication
6– 8–29	7.2	5.6	367	21.5	670			External genitalia still enlarged Metoestrum
6–23–29								Animal appears ill Anorexia. Decision to give calcium by stomach tube Animal developed diaphragmatic spasm and died in half hour of respiratory failure
								Dog 8 Parathyroidectomy 1–10–29 Last tetany 2–7–29
5–21–29	11.7	4.5	338	26.1				2nd day proestrum
5–27–29	5.4	6.0	351	34.0	615			3rd day oestrus No tetany but anorexia
5–29–29	5.8	6.1	342	25.2	630			5th day oestrus No tetany
6– 3–29	5.6	4.9	361	24.6	662			Metoestrum
6–11–29	7.0	5.4	356		629			Had developed "snuffles." Dead 6–15–29 in depression

had been taken. Successful copulation with a parathyroidectomized male was observed, after which the female dog developed a severe attack of tetany. (Blood calcium 9.8, phosphorus 6.0.) Calcium gluconate was given by intravenous injection and the dog recovered. By 6–25–29 it was evident the dog was pregnant and to our surprise the blood sample showed a normal serum calcium. No signs of tetany were present thereafter, despite the lowered serum calcium on 7–26–29. During an experiment on vaginal motility on 8–25–29 the dog aborted a 13 cm. fetus; no tetany was observed before or after this abortion.

Dog 7 (not considered in table 1) died on the second day of oestrus (with serum calcium 6.1, phosphorus 8.6) after a violent attack of tetany. A week before, on the first day of proestrum, the serum calcium was 8.0 and phosphorus 4.9. It would appear in this instance that the drop in serum calcium and rise in phosphorus were important factors in this

tetany. However, there is the case of dog 8 (table 1) who had on the third day of oestrus a serum calcium 5.4, phosphorus 6.0, a change from the proestrous figures of calcium 11.7, phosphorus 6.0 and yet no tetany occurred during oestrus.

Mathieu and Barnes (1933) demonstrated that when theelol or theelin is administered to parathyroidectomized female dogs, a fall in serum calcium occurs, the lowered level persisting throughout the injection period. They found no correlation between the drop in serum calcium and tetany in "artificial" oestrus and observed only one attack of tetany with this drop in serum calcium. Their study indicates that in parathyroidectomized female dogs, a low serum calcium may be further lowered by female sex hormone without precipitating tetany.

In conclusion, then, it may be said that our data indicate that in the parathyroidectomized female dog in natural oestrus in the latent tetany period, tetany can occur with either a further drop in serum calcium or when the serum calcium level is maintained at the anoestrous level; we would conclude, then, that the occurrence of tetany in oestrus in the parathyroidectomized dog is not dependent upon a further lowering of the possibly already low serum calcium. Changes in serum phosphorus, sodium, potassium, or chlorides (and probably pH and CO_2) are probably not involved in the severe tetany of oestrus in parathyroidectomized female dogs.

Those who have closely observed unoperated female dogs in oestrus are well aware that the bitch at this time is often highly excited and restless. The neuromuscular balance of the parathyroidectomized dog is easily upset and it is altogether possible that the greatly increased nervous discharges during oestrus would so upset this balance as to throw the animal into tetany. Our impression is that the cause of this tetany of oestrus in parathyroidectomized dogs is linked more closely to an instability of the nervous system rather than to changes in ionic balance in the blood stream at oestrus.

SUMMARY

There is no apparent change in the blood values for several inorganic ions and pH and CO_2 of normal female dogs during the oestrous cycle. Tetany can occur in the parathyroidectomized female dog coming into natural oestrus in the latent tetany period with either a comparatively normal or low serum calcium. It appears that other physiological changes are responsible for this type of parathyroid tetany.

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CARDIOVASCULAR REACTIONS INDUCED BY ELECTRICAL STIMULATION OF THE CEREBRAL CORTEX[1,2]

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The existence of cortical influence upon the cardiovascular system has been indicated in part by observations of heart rate and blood pressure changes in patients with known cerebral lesions (Minkowski, 1; Popper, 2), and in subjects capable of voluntary cardiac acceleration (Taylor and Cameron, 3). Such influence has been further suggested by clinical observations of localized disturbances of vasomotor activity in cases of hemiplegia beginning with work of Chevallier in 1867 (4), to which attention has recently been called by Fulton (5); and in studies on animals with experimental lesions of the cortex. (See Pinkston, Bard and Rioch (6), and Kennard (7).) Additional evidence has also been adduced from the results of electrical stimulation of the surface of the cerebral hemispheres. Studies of the latter type began with the discovery by Schiff (8) that the heart rate could be altered, and by Danilewsky (9) who pointed out that the blood pressure could also be changed. In spite of the fact that these findings have been repeated several times, Bard (10) has felt "that many of the effects which were produced are to be accounted for on the basis of spread of strong stimulating currents to subcortical regions or to production of cortical epilepsy in which a widespread and unphysiologic discharge occurs." Thus, although clinical and physiological literature contains the implication that the cerebral cortex plays a part in the regulation of cardiovascular functions, nevertheless such cortical influence has neither been completely established nor universally accepted. The investigations to be reported in the present paper are an attempt to elucidate this relationship in controlled stimulation experiments.

METHODS. This report is based on a study of 40 cats, 18 monkeys (Macaca mulatta) and 1 chimpanzee anesthetized with ether. The cerebral cortex was stimulated by a Harvard inductorium, a thyratron tube discharging a condenser through a

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[2] Preliminary reports of these experiments were communicated to the Yale Medical Society, November 13, 1935 (Yale J. Biol. Med. **8**: 201, 1935), and to the American Physiological Society, March 26, 1936. (This Journal, Proc. 1936.)

loosely coupled step-up transformer or the standard 110 volt 60 cycle alternating current reduced to the proper voltage by means of a potentiometer. In most cases simple bipolar electrodes were used with the tips separated approximately one millimeter. Blood pressure was recorded in most instances from the femoral artery by either a mercury manometer or a Wiggers optical manometer. Pupillary changes were observed. In many experiments a slow intravenous infusion of saline or Locke's solution was maintained. In a few cases epinephrine was added to this perfusion fluid in the proportion of 2 to 4 drops of a 1:1,000 solution to 250 cc. of perfusion fluid. Since a finer adjustment of stimulus intensity was required to differentiate cardiovascular responses from skeletal muscular movement than was usually convenient, the expedient was adopted in the later experiments of injecting sufficient curare to prevent contractions of the skeletal muscles.

RESULTS. 1. *Changes in blood pressure.* In cats and monkeys anesthetized with ether stimulation of the motor region of the cortex regularly produced a rise in blood pressure after a latent period of 1.5 to 10 seconds followed by a return to normal beginning approximately the same number of seconds after the cessation of stimulation. With a prolonged stimulation the pressure reached a peak after 10 to 20 seconds and then began to decline slowly, the rate of fall usually being accelerated by cessation of stimulation. Associated with the rise in pressure, there was often an acceleration of the heart of 5 to 10 per cent, while a slowing of as much as 50 per cent often occurred during the post-stimulatory decline of arterial pressure. Records illustrating rise of pressure are reproduced in figures 2 to 6. In all records the points of stimulation are indicated by a numbered dot on the diagram accompanying the record.

In one chimpanzee, anesthetized with ether and in the latter part of the experiment curarized to prevent muscular movements, a rise of pressure amounting to 10 to 20 mm. Hg has been elicited by stimulating the motor and premotor cortex in the region adjacent to the superior precentral sulcus and the posterior part of the superior frontal sulcus using the A.C. 60-cycle current at a strength of 3 to 4 volts. These pressor responses were usually associated with muscular activity and occasionally with epileptiform contractions. However, the rise in pressure usually preceded the muscular activity, beginning after a latent period of 3.5 to 5.0 seconds and commencing to decline within 4.0 seconds after cessation of the stimulus. The return to pre-stimulation level was complete in 25 seconds. The rises in blood pressure were greater after abolition of muscular responses with curare and were evoked with a lower current strength than before curare was administered. This facilitation of pressure rise from cortical stimulation following the administration of curare has also been observed in experiments on cats and monkeys.

Various points on the parietal cortex were stimulated in 11 cats anesthetized with ether. No alteration of blood pressure occurred in 5 of these, but in 6 of them a decline of pressure of 10 to 15 mm. Hg followed

stimulation of appropriate points in this area. In many instances of maintained excitation this fall was succeeded by a rise. In the monkey a decline of pressure was not as commonly observed as in the cat but the blood pressure was sometimes diminished during stimulation of the marginal gyrus and a small area of the cortex near the anterior tip of the superior precentral sulcus (fig. 1, C and D). A typical fall of pressure in a cat is illustrated in figure 2, record 53.

2. *Changes of heart rate.* The cortex on and adjacent to the gyrus proreus was stimulated in 5 cats deprived of both stellate ganglia and both adrenal glands. No change in heart rate was observed in two of them, but in 3 of these animals the heart was slowed by as much as 50 per cent. A similar slowing of the heart was obtained from a point 1 cm. anterior to the superior precentral sulcus in one monkey on which the same operative procedures had been performed. In all cases the slowing was asso-

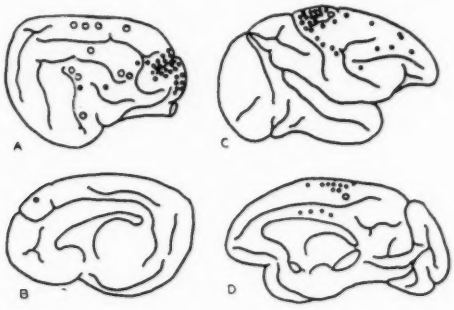

Fig. 1. Diagrams of the pressor and depressor points. A, lateral and B, medial surface of the cat's cortex; C, lateral and D, medial surface of the monkey's cortex. Solid dots—rise of pressure; circles—fall of pressure.

ciated with either no change or a fall of pressure. Since these changes in heart rate have been only occasionally observed, it has not been possible to determine definitely whether they are the result of vagal stimulation or sympathetic inhibition, but, since they were obtained in stellatectomized, adrenalectomized animals, the slowing would appear to have been primarily a vagal effect.

3. *Other autonomic phenomena.* In both cats and monkeys, dilatation of the pupils, and in cats, retraction of the nictitating membranes occurred simultaneously with the rise in pressure obtained from the gyrus proreus and the anterior sigmoid gyrus (cat) and the area just above the superior precentral sulcus (monkeys). The pupils and nictitating membranes returned to the basal state as the pressure fell. Gastric responses were not studied.

In one monkey (C.S. 34) distinct elevation of the hair on the dorsum

of the shoulders and upper back was obtained from stimulation of points on the cingulate gyrus corresponding to the four dots placed in this area in figure 1 D.

4. *Cortical areas from which cardiovascular responses were obtained.* In the cat under ether anesthesia the vasopressor responses, indicated by solid dots in figure 1, A and B, were obtained most regularly from the posterior and anterior sigmoid gyri and the adjacent part of the gyrus proreus. Occasional pressor points were also located on the anterior portions of the suprasylvian and ectosylvian gyri and on the anterior lip of the sylvian fissure. As figure 1 indicates, elevations of pressure were also recorded during stimulation of a point on the marginal gyrus on the medial surface of the hemisphere. Depressor points, indicated by circles, were consistently located in the cat on gyrus lateralis medius, gyrus suprasylvius medius, gyrus ectosylvius medius and gyrus sylvius.

Rises in pressure were most readily obtained in the monkey anesthetized with ether from the cortical areas adjacent to the superior precentral sulcus (fig. 1, C and D). Vasopressor responses also occurred during stimulation of the trunk and arm areas and the superior, middle, and

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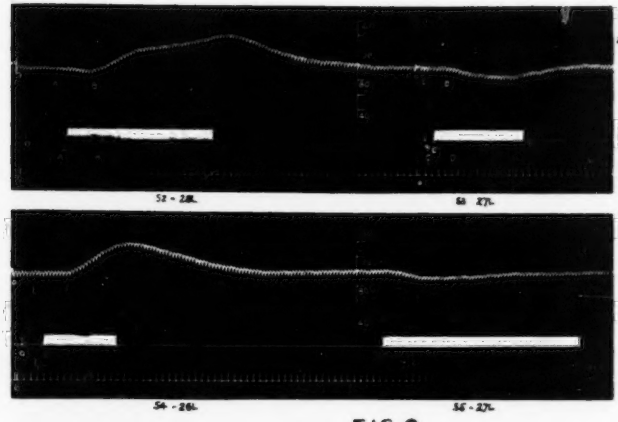
Fig. 2. A consecutive series of records illustrating the close proximity of pressor and depressor points in a cat. Male, weight 3.0 kgm., ether anesthesia, curare 45 mgm., Harvard inductorium 8.5 cm., artificial respiration, saline infusion. Record 52, a rise in pressure obtained from point *28* just in front of the anterior tip of the lateral sulcus; record 53, a fall of pressure due to stimulation of point *27* lying on gyrus supra sylvius anterior 3 mm. lateral to point *28*; record 54, a rise of pressure from stimulation of point *26* on the gyrus suprasylvius anterior, posterior to point *27*, and approximately 3 mm. from points *27* and *28*; record 55, stimulation of point *27*. Experiment C. S. 23.

Fig. 3. Consecutive records illustrating absence of current spread in monkey. Female, *Macaca mulatta*, weight 3.5 kgm., ether anesthesia, curare 10.5 mgm., Harvard inductorium 12 cm. angle 20 degrees from horizontal, artificial respiration, saline infusion. Record 29, stimulation of point *9* lying on left leg area 1 mm. anterior to central sulcus; record 28, stimulation of point *18* 1 mm. posterior to the central sulcus and not more than 3 mm. distant from point *9*. Experiment C. S. 32.

Fig. 4. Records demonstrating that blood pressure responses are abolished by local anesthesia of the cat's cortex to a depth of 3 mm. Female, weight 2.9 kgm., ether anesthesia, curare 20 mgm., Harvard inductorium 8.5 cm., artificial respiration, saline infusion. Records 12 and 13, stimulation of points *5L* and *5R* on the left and right posterior sigmoid gyri. Immediately after the record 13, a 2 per cent solution of Nupercaine hydrochloride N.N.R. was applied by means of a small pledget of filter paper to the right cortex in the region corresponding to point *5R*. Records 30 and 31, stimulation of points *5L* and *5R* 32 minutes after beginning the application of Nupercaine to *5R*. Record 32, stimulation of point *5R* with the electrodes pushed 3 mm. into the cortex.

Fig. 5. Pressure rise unaccompanied by skeletal muscular movement in a non-curarized cat. Male, weight 3.0 kgm., ether anesthesia, Harvard inductorium 7.5 cm. Experiment C. S. 16-10-3R.



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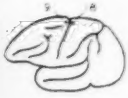

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FIG. 2

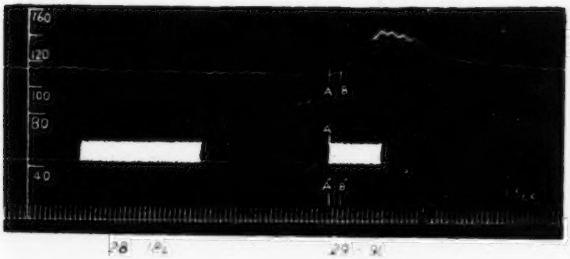

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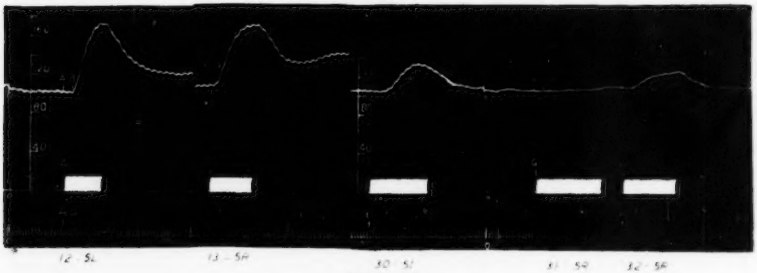
FIG. 3



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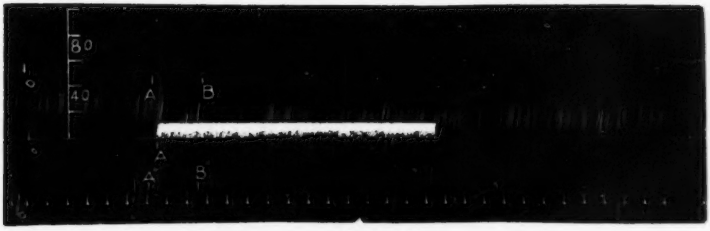

FIG. 5

Figs. 2-5

inferior frontal gyri. Points on the marginal and cingulate gyri on the medial surface, anterior to the central sulcus also gave pressor responses. The areas giving a fall of pressure have already been indicated.

Although other surfaces of the cortex have been explored, no additional points have been found which produce vasomotor changes. Thus in the monkey, the postcentral gyrus in particular as well as many points on the parietal lobe, the occipital lobe, and the upper half of the temporal lobe all failed to give vasomotor responses. The orbital surfaces of the

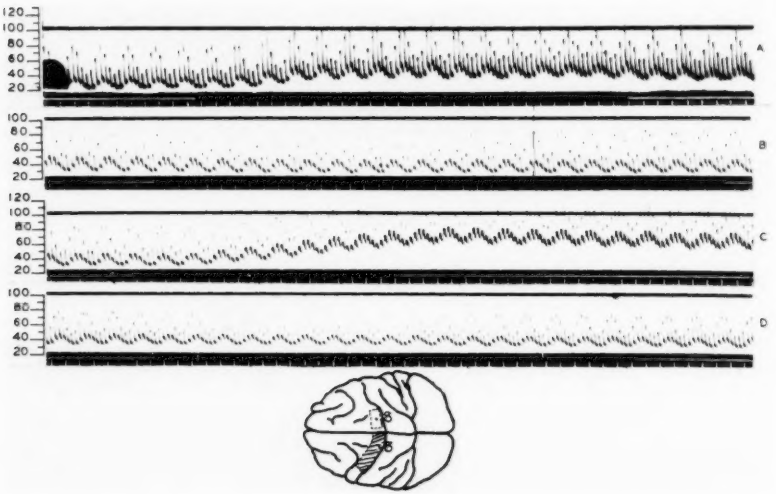

Fig. 6. Demonstration of the localization of the stimulated cells within the cortex of the monkey. Female, *Macaca mulatta*, weight 4.65 kgm., left area 4 (Vogt) removed 109 days before, ether anesthesia, curare 18 mgm., thyratron stimulator—60 cycles per second 150 peak volts, artificial respiration, A-28—normal response from point *8R* on right cortex; B-30—absence of response from point *8R* after undercutting; C-31—response from white matter exposed by removal of a block of cortex including point *8R*; D-32—absence of response from point *8L* in white matter exposed by previous ablation of left cortex. Experiment C. S. 42.

hemispheres were not explored nor were the medial surfaces with the exception of the point to which reference has already been made.

An examination of microscopical sections of the responsive areas of the cat's cortex has demonstrated that vasomotor responses were obtained from areas in which large pyramidal cells were present (areas B, E and F of Langworthy, 11, 12; and the motor cortex of Campbell, 13) as well as from areas in which they did not occur (area A of Langworthy and frontal cortex of Campbell).

5. *Demonstration that responses are due to the stimulation of nerve elements*

within the cortex. The above demonstrated cardiovascular responses resulting from the application of electrical currents to the cerebral cortex may have been due to the excitation of subcortical (e.g., hypothalamic) centers by spread of current from the points of stimulation, they may have occurred as a reflex response to stimulation of nerve fibers lying in the dura or accompanying the blood vessels of the cortex, or finally they may have been due to the activation of specific nerve elements within the cortex itself. That the first two possibilities cannot explain the changes in vascular tension and cardiac activity recorded, and that these findings may validly be considered the result of excitation of a nervous mechanism specifically localized within the cerebral cortex is suggested in the following three lines of evidence:

a. *Pressor and depressor points can frequently be discretely localized within 2 to 4 mm. of each other.* An experiment demonstrating this fact is illustrated in figure 2, taken from records obtained from the cat. Similar observations have been made in the monkey as illustrated in figure 3. In the latter example the path for the spread of electrical currents between the points was very short, although the points were separated some distance anatomically, while in the experiment illustrated in figure 2 the points were also very close together anatomically. Were the responses due to stimulation of nerves accompanying cortical blood vessels, such responses should always be the same and should be obtained from all parts of the cortex. It may be concluded, therefore, that the strength and manner of application of stimulation precluded spread of current of effective strength to adjacent cortical areas, to blood vessels, to dura or to subcortical structures.

b. *Local application of anesthetics to an excitable area of the cortex abolishes the responses from this point.* Records illustrating this phenomenon are reproduced in figure 4. Since the anesthetic abolished the response to stimulation of the cortex, and since the anesthesia extended only three millimeters into the depth of the hemisphere, it would seem reasonable to conclude that the responses must have been due to the stimulation of nerve elements lying within the outer three millimeters of the cortex. This experiment provides further evidence that current spread to subcortical centers plays no part in the vasomotor responses obtained.

c. *The response from a given point is abolished by undercutting this area of the cortex.* This phenomenon is illustrated in figure 6. That the rise of pressure was produced by stimulation of descending fibers from the cortex is shown by a comparison of record C with record D obtained while stimulating a corresponding point on the left hemisphere. This latter point lay on the white matter which had been exposed by removal of left area 4 sufficiently in advance to assure complete degeneration of the descending fibers. Since stimulation of point *8* on the left side pro-

duced no change of blood pressure it is presumable that the rise of pressure from stimulation of the white matter on the right side was due to the excitation of axones whose cell bodies lay within the cortex of area 4. Such axones while excitable on the right side (acute preparation), were degenerated on the left side (chronic preparation). Similar procedures have been carried out in the cat with the same results.

6. *Differentiation of vasomotor and somatic muscular responses.* François-Franck (14) and recently Mettler, Hamilton, Allen, Woodbury and Mettler (15) have stated that blood pressure changes from cortical stimulation are usually associated with an epileptiform seizure. François-Franck, in fact, never obtained cardiovascular responses from the cortex at a distance from the motor area except with stimuli which also provoked an epileptic attack. The rises in pressure accompanying these seizures were always of long duration, and since the blood pressure response to strong stimuli in the deeply curarized animals was entirely similar to that accompanying the epileptic attack in the non-curarized animals he suggested that while curare had abolished the motor manifestations of the seizure, the visceral component persisted (p. 188), and that the epileptic state was thus reduced to what he termed its organic or internal manifestations. As he stimulated nearer to the motor area simpler, briefer reactions appeared and with moderate stimuli applied to the motor cortex itself he obtained simple cardiovascular changes of brief duration unassociated with an epileptic state (p. 207).

Although we have obtained blood pressure changes associated with epileptiform discharges of cortical origin in our own experiments nevertheless it has been definitely possible to demonstrate specific alterations of blood pressure of brief duration unaccompanied by somatic responses. For example, in experiments 14 to 20 elevation of pressure was repeatedly obtained in cats lightly anesthetized with ether and without curare, using strengths of stimuli which produced no movement whatever. Figure 5 shows a typical record from one of these experiments. Furthermore we frequently noted as a result of cortical stimulation vigorous somatic movements of an epileptiform type which were unaccompanied by any alteration in blood pressure.

Discussion. The observations reported in this paper conform in general to those of previous workers (see Weber, 16; Bechterew, 17; Dusser de Barenne and Kleinknecht, 18; Hunsicker and Spiegel, 19, and others quoted by these writers), who have all reported that either a rise of pressure or alterations of heart rate or both can be produced by cortical stimulation. That a fall in pressure can be consistently produced by cortical stimulation in the cat and dog was demonstrated by Dusser de Barenne and Kleinknecht (18). This finding has been confirmed in the cat under ether anesthesia in which, however, the points giving a fall in pressure

were restricted to the lateral, suprasylvian and ectosylvian gyri. It has not been possible under ether anesthesia to produce a consistent fall of pressure in the monkey.

In agreement with earlier observers the latency of the blood pressure responses to cortical stimulation has been found to be on the average 3.5 seconds. These short latent periods suggest that the cortical mechanism acts primarily through nervous channels. This suggestion is further supported by the fact that blood pressure rises still occurred after removal of the adrenal glands. Although in some of the experiments where the stimulation was continued for as long as 40 seconds the pressure reached a peak and began to decline on the average 16 seconds after the beginning of stimulation possibly as a result of fatigue, nevertheless it must be emphasized that in those experiments in which minimal stimuli were used the pressure always began to return toward normal within 2 to 4 seconds after cessation of stimulation and had usually reached the prestimulation level within 15 seconds. In instances in which excessive current strength was used the pressure did not fall at the end of the stimulation interval and frequently continued to rise further for 30 seconds to 2 to 3 minutes. This phenomenon is probably due to persistence of cortical activity, and may be referred to as after-response in the cardiovascular system.

In view of the evidence presented it must be concluded that there are nerve pathways from the cortex capable of altering the degree of vascular constriction and thus of causing a change of blood pressure. Whether these corticofugal pathways mediate their effect via the hypothalamus or whether they act directly on lower centers cannot at present be decided.

The location of the pressor and depressor points in cats confirms the general localization of these points by previous observers. In the monkey, vasopressor points have not previously been plotted. Although Schäfer (20) refers to some unpublished experiments in which he and Bradford observed vasodilatation resulting from stimulation of the cerebral cortex of monkeys in the region of the gyrus marginalis and gyrus fornicatus and vasoconstrictor effects from other regions of the cortex which were not specifically noted. These findings of vasopressor points in the motor area of the cortex have recently been confirmed in preliminary notes by Mettler *et al.* (15), and by Crouch and Thompson (21).

The epileptiform motor after-response which follows stimulation of the cortex with excessive strength of stimulation is well-known. That a visceral type of epileptiform after-response can also be initiated from the cortex has been demonstrated by certain experiments in which excessive strengths of stimuli, deliberately used, resulted in the persistence of a continued rise or alternate rise and fall of pressure lasting from 30 seconds to 2 or 3 minutes after cessation of stimulation. Since the visceral after-

responses have been obtained in etherized animals and also in animals in which all muscular response has been abolished by curare, these phenomena cannot be due to a delayed effect of muscular contraction. Furthermore when the strength of stimulation is reduced, a normal rise of pressure still occurs, but, on discontinuing the stimulation, pressure does not remain elevated but rapidly returns to the pre-stimulation level. Therefore it seems probable that the after-response in the cardiovascular system is due to the abnormal persistence of activity within the cortex after removal of the stimulus.

The results of these experiments clearly demonstrate the existence of two distinct cortical mechanisms. The first of these exerts control over somatic movement as shown by the normal motor response to stimulation and the exaggerated activity of the epileptiform type of motor after-response. The second influences vasomotor and other visceral activities, and is demonstrated by the occurrence of heart rate and blood pressure changes during cortical stimulation in the absence of any somatic activity. Since the alterations in the vasomotor mechanism which would reasonably be expected to occur under cortical direction would be those concerned in adjusting the responses of the vascular system to the activities of the somatic, it is not surprising that the distribution of the representation of the vasomotor system so closely overlaps that of the somatic. In view of this close anatomical association it is quite possible that in the normally functioning individual afferent impulses which activate the somatic efferent will also affect the visceral efferent mechanism.

The abnormal visceral activity which precedes and accompanies an epileptic attack has been described by Frisch (22), and furthermore according to Watts and Frazier (23) such phenomena evidently occur in the absence of skeletal motor manifestations. Thus the cortical lesion responsible for the somatic manifestations of the clinical epileptic seizure may also initiate activity within the overlapping cortical visceral mechanism, and so account for the vegetative disturbances which precede and accompany the attack.

SUMMARY

1. These investigations were undertaken to verify by controlled cortical stimulation experiments the evidence for a cortical influence on the cardiovascular system.

2. Blood pressure and heart rate were recorded by mercurial or optical manometers during stimulation of the cerebral cortex of 40 cats, 18 monkeys and 1 chimpanzee under light ether anesthesia, and in many instances curarized to prevent muscular movements.

3. From the gyrus proreus and the sigmoid gyri in cats and the cortex adjacent to the superior precentral sulcus as well as scattered points in

the trunk and arm areas and on the superior, middle and inferior frontal gyri, and marginal and cingulate gyri anterior to the central sulcus in monkeys, rise of blood pressure, acceleration of heart rate, retraction of the nictitating membranes and dilatation of the pupils were recorded.

4. A decline of pressure frequently resulted from stimulation of the lateral, suprasylvian, ectosylvian and sylvian gyri in cats and occasionally the marginal gyrus and a small area of cortex near the anterior tip of the superior precentral sulcus in monkeys.

5. Marked slowing of the heart occurred in a few instances of cortical stimulation especially in animals from which the stellate ganglia and adrenal glands had been removed.

6. That these cardiovascular responses were initiated by stimulation of nervous elements situated within the cortex may be concluded on the basis of four lines of evidence: *a*, pressor and depressor points were discretely localized within 3 to 4 millimeters of each other; *b*, local anesthesia of the cortex to a depth of three millimeters abolished the responses; *c*, isolation of a small responsive area of the cortex by undercutting eliminated responses from this area; *d*, excitation of the efferent fiber tracts in the white matter immediately below the cortex normally gave a vasomotor response but none resulted from stimulation of these tracts when they had become degenerated as a result of previous removal of the overlying cortex.

7. These responses are not a part of an epileptiform muscular response since they can be elicited in non-curarized animals in which no muscular movement has occurred, and furthermore epileptiform type of muscular responses have been observed without change in blood pressure.

8. It is therefore concluded that there is a mechanism by which the cortex can influence the state of the cardiovascular system, and that through this mechanism the cortex may bring about a finer adjustment of the activity of the heart and circulation in accordance with the exigencies of the external environment and the immediate activities of the skeletal musculature. The existence of such a mechanism may explain the origin of the disturbances of the heart and circulation associated with the epileptic seizure.

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STRUCTURAL AND FUNCTIONAL ORGANIZATION OF THE CENTRAL MECHANISM CONTROLLING BREATHING[1,2,3]

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Probably the first recorded opinion on the localization of the control of breathing is that of Galen who reported instantaneous death from section of the cord at the level of the first and second vertebrae. The same effects were again noted by Lorry in 1760 on dividing the cord between the second and third vertebrae. Legallois (1812) then designed transection experiments for more precise localization of respiratory control and concluded that breathing is dependent upon a particular and small part of the medulla near the origin of the pneumogastric nerve. These experiments mark the beginning of an augmenting accumulation of valuable, but often conflicting, data on a most involved subject.

The need of more exact information on the localization of the central controlling mechanism of breathing and on the structural organization and function of its component cell groups scarcely requires comment. To fully understand the train of events leading to a coördinated respiratory act, it is quite essential to follow the process from the sensory receptors, through the central nervous system, and to the motor end organs, for until we know the units participating we cannot study effectively the processes involved. The electrical method which tells where and how a cell is acting seems to offer a direct and supplementary attack.

METHOD. Four phases of this problem have required our special attention: the finding of respiratory potentials; the registration and study under various respiratory conditions; the histological localization of the source of potentials; and the interpretation of results.

Finding respiratory potentials. Our experiments were done on dogs anesthetized with morphine and urethane or with evipal.[4] The skull cap was resected and in most of the experiments the cerebral hemispheres and

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[4] This anesthetic was supplied by Winthrop Chemical Co., New York, N. Y.

portions of the cerebellar vermis were removed by suction. In the experiments, confined mainly to the study of potentials in the mesencephalon and diencephalon, where evipal was used, we may assume little or no chemical anesthesia throughout our observations, but in the experiments in which morphine and urethane were employed we must assume chemical action of the anesthetics after, as well as before, decortication.

The exposed brain stem was explored for respiratory potentials with two fine insulated needle electrodes, bare at the tips, separated 1.5 to 2 mm. along the longitudinal axis of the animal. The electrodes were mounted on a specially constructed and calibrated manipulator, allowing movement in the longitudinal and transverse directions and penetration in the dorso-ventral direction. The electrodes were connected, through amplifiers, with a loud speaker for auditory detection and with a General Electric Multiple Oscillograph for visual inspection and registration of potentials. The dogs were placed in the prone position with the head firmly fixed in a specially devised head holder to avoid artefact potentials of mechanical origin. Electrical interferences were avoided when necessary by shielding with an adjustable aluminum shield.

With graded penetration there was an ever changing medley of action sounds—some weak, others loud; some discrete, others indiscrete, i.e., out of phase with one another; some of regular frequency, others of irregular sequence; some of high frequency and some of low frequency; and some fluctuating with the respiratory rhythm.

We may assume with a fair degree of certainty that potentials showing fluctuations in rhythm with respiration are in some way related to respiration. On the other hand, we cannot conclude with equal certainty that potentials failing to display such fluctuations are unrelated to respiration. This constitutes, for the time, a more uncertain angle of the problem.

In our earlier experiments we studied only potentials showing respiratory rhythm. In searching for these potentials we adopted the procedure of inserting the electrodes at 1 mm. intervals along parallel and transversely directed lines 2 mm. apart. With each penetration from the dorsal to the ventral surface, the electrodes were inserted at steps of 0.1 to 0.2 mm. and after each advancement of the electrodes an interval of several respirations was allowed for the auditory detection of respiratory potentials. A complete exploration of the medulla and upper cord was slow and tedious, yet a thorough exploration seemed quite essential. First of all, the electrodes are highly selective and tap potentials only in their immediate vicinity, thus unless cells are discharging en masse they could easily be missed; secondly some of the structures supposedly participating in the respiratory act are very small; and thirdly respiratory potentials at best were relatively hard to find.

When respiratory potentials were encountered they were photographed

along with changes in tracheal pressure and tidal air. Time was recorded in 0.04, 0.20 and 1.0 second intervals and in many experiments mean blood pressure and the pulse were registered as well (see figs. 2–7). The procedure is similar to that previously described in studies on the action potentials of respiratory muscles (Gesell, 1936a). Downstroke represents inspiration in both tracheal pressure and tidal air records and upstroke represents expiration. The tracheal pressure record gives the change in phase of breathing with accuracy but the tidal air tracing shows considerable lag.

After having completed any set of observations at a single setting of the electrodes, a lesion was placed by passing a momentary weak galvanic current across the electrodes at the site of the potentials. Each lesion was entered in the protocol with respect to gross position determined by the three dimensional manipulator to insure later positive histological localization. At the close of each experiment the dog was killed, the brain stem was carefully removed and fixed in trichloracetic acid, mercuric chloride and alcohol, embedded in paraffin, and cut into 20 micra sections. Every fifth section was mounted in serial order and stained in toluidin blue according to the method described by Huber (1927).

The lesions were approximately 0.5 mm. in diameter. Occasionally they were difficult to find due to small hemorrhages from the rupture of minute blood vessels by the needle electrodes. In such doubtful cases of identification experimental findings were disregarded. The highly expert judgment of Doctor Crosby on the possible nervous structures involved in the lesions gives us confidence in the anatomical phase of our problem.

Gross localization of respiratory potentials. We have studied the earlier experiments of others and have presented condensed discussions, under appropriate headings, of those results which seem essential to the interpretation of our findings. These discussions may be followed to better advantage by reference to our sketch of the medullary nuclei (fig. 10) as constructed from serial sections of the brain stem of the dog. A more extensive review, including the higher "respiratory centers" is available in the excellent discussion *Le Centre Respiratoire* by Cordier and Heymans (1935). See also the recent review by Finley (1931).

Since the transections of the brain stem by Legallois abundant evidence has accumulated indicating that the primary control of breathing resides in the medulla. Charles Bell (1833), mainly from clinical observations, concluded that "In the lateral portion of the medulla oblongata, from which the several respiratory nerves go off, there is seated a power which passing through the diverging nerves combines the remote organs." Flourens (1842–51), with experimental methods, limited the center to his "noeud vital" near the obex of the medulla. Longet (1847), Mislawsky (1885), Laborde (1890), Gad and Marinescu (1892), Arnheim (1894), Kohnstamm (1900), Bechterew (1908), Finley (1931), Johnston (1932)

all placed the seat of control in the medulla. Their experiments will be reconsidered in relation to the formatio reticularis. Schiff (1858), Gierke (1873) and Girard (1890) reached similar conclusions. Their findings will be reconsidered in connection with the alae cinereae.

Kronecker and Marckwald (1880) and Marckwald (1887) transected the medulla at the cephalic end without noticeable effects on breathing. At a decapitation (between the 4th and 5th vertebrae) Gad in 1886 (quoted from Kronecker, 1887) saw the trunk fall limply while the head made dyspneic movements for 1½ minutes. Paralysis of breathing by cooling (Fredericq (1883), Trendelenburg (1910), and Nicholson (1936a)) and by local application of cocaine hydrochloride to the floor of the fourth ventricle (Aducco (1890), Franck (1892), Langlois and Garrelon (1908), and Nicholson (1936b)) indicated that the primary respiratory center resides in the medulla and dominates the cord.

Grossmann (1889) proposed a reciprocal association of several respiratory centers as opposed to a complete subordination of various centers to a primary medullary center. According to Trevan (1916) the respiratory center is a chain of neurones extending along the entire floor of the fourth ventricle and removal of any part affects coördinated breathing. He suggested with Boock that while the medullary center is primitive, complete dependence of breathing on the vital node must be sought in some animal lower than the frog. Springer (1928) believes that the selachians possess a central motor mechanism of respiration located in the medulla. Hyde (1894) concludes that each ganglion of the cord of the Limulus constitutes an automatic and reflex center for the respiratory movements; that lesion or loss of part of the entire brain, esophageal collar, or post-esophageal ganglion has no influence on the respiratory movements; that after severing the cord the anterior and posterior divisions show the same respiratory response to exercise, feeding, and irritation. She finds a higher development of the respiratory center in the skate (1904), intermediate between the simple segmental arrangement of invertebrates and the complex modified and specialized centers existing in higher vertebrates. According to her the respiratory center of the skate occupies definite sensory and motor areas in the medulla; the movements are segmental but the relationship of the respiratory organs and their segmental centers is not so obvious as in the lower forms; the developmental changes of shifting and consolidation have begun to mask the segmental connection of the brain; and where development has proceeded a step further this connection would be demonstrated only with difficulty.

The recent findings and conclusions of Lumsden (1923) are particularly interesting in relation to this concept of increasing complexity of the respiratory mechanism with evolutionary development. Modifications of breathing produced by transection of the brain stem of the cat led Lumsden

to postulate four highly specialized centers. A gasping center below the striae acusticae provides the primitive respiratory act composed of a deep, short, active inspiration followed immediately by a passive expiration. This act is in turn modified by higher centers. The most caudal of these centers is the expiratory center which adds active expiration. Next comes the apneustic center which prolongs the period of inspiration producing apneustic breathing and finally the pneumotaxic center which periodically interrupts apneusis and produces normal rapid breathing.

Henderson and Sweet (1929) attributed Lumsden's results to involvement of the rubro-spinal tract. Keller (1931) disagreed with Henderson and Sweet. Nicholson (1936a) states "The fact that a marked apneusis may be brought about by a moderate cooling of a small area in the region of the calamus scriptorius seems at least to remove the necessity for the existence of a pneumotaxic center in the upper pons with the specific function of interrupting inspiration. It appears that inspiration is normally rhythmically interrupted by the activity of some mechanism located just anterior to the apex of the calamus scriptorius." To these various criticisms may be added that of Barcroft (1934) who has accepted the gasping center as an established fact but explains apneustic breathing by a reduction of normal proprioceptive impulses which automatically function to cut each inspiration short. In more recent experiments, Henderson and Craigie (1936) again fail to confirm the findings of Lumsden and conclude from a series of varied lesions that the respiratory area appears to lie in the medial third of the medulla, limited above approximately at the junction of the middle and upper thirds of the hypoglossal nucleus and below by the pyramidal crossing.

We have done some eighty experiments dealing with the localization of the central respiratory mechanism by means of electrical sounding. In sixty of these, the explorations were confined to the medulla and upper cervical region of the cord between transverse sections 12 mm. above and several centimeters below the obex. In the remaining twenty, the explorations were limited to the thalamus, hypothalamus, mid-brain, pons and medulla above a level 8 mm. cephalad to the obex. A few explorations were made in the cerebellum but none were made above the thalamus.

The results of the first group of experiments are presented in figures 1A and B in which the location of both inspiratory and expiratory potentials are plotted to scale. Inspiratory potentials are designated by an *O* and expiratory by an *X*; where both occurred along one penetration of the electrodes they are designated by an *X* enclosed in an *O*. The medial and lateral reticular nuclei are also plotted in figure 1A as accurately as their diffuse structures will permit and the accompanying figure 1B shows the important respiratory nuclei plotted to the same scale. Neither the depth of potentials nor the depth of the nuclei is indicated. The histological localiza-

TABLE 1

Tabulation of structures involved in lesions associated with respiratory and continuous potentials

	INSPIRATORY	EXPIRATORY	CONTINUOUS
Brachium conjunctivum	49/4 (I-5)		
Descending root of V	14/8 (P-8)		
Nucleus of	51/2 (J-2)		
Dorsal funiculus of spinal cord	57/2 (G-3)	19/4 (C-2)	
		22/2 (A-3)	
Dorsal root fibers	19/1 (B-1)	19/1 (B-1)	23/2
		57/3 (H-5)	
Collaterals of	15/2 (C-1)	15/2 (C-1)	
	21/3 (A-2)	37/1 (H-4)	
Commissural fibers from	49/1 (I-3)		
Dorsal root fibers and edge of dorsal funiculus		22/3 (A-4)	
Fasciculus solitarius			28/4
			56/4
Nucleus of	27/6 (N-4)		
	12/1 (P-5)		
Fibers from nucleus to dorsal efferent Nucleus of X	36/4 (O-4)		
Internal arcuate fibers	27/1 (S-1)		
(These lesions fall on prominent bundles of arcuate fibers)	46/1 (O-5)		
	47/3 (M-2)		
	48/3 (L-2)		
	50/1 (M-4)		
	50/3 (N-5)		
	51/1 (J-3)		
	55/1 (O-6)		
	57/5 (M-6)		
Lateral funiculus of spinal cord	56/2 (I-1)		
Lateral reticular nucleus	50/4 (N-6)		
Lateral reticular nucleus and internal arcuate fibers	47/5 (O-7)	18/4 (T-2)	
Lateral reticular nucleus and lateral reticulo-spinal tract	56/5 (M-7)		
Lateral reticulo-spinal tract	14/1 (D-1)	22/1 (A-1)	
	19/2 (B-2)	49/3 (J-5)	
	19/5 (C-3)		
	19/6 (D-4)		
Lateral reticulo-spinal tract and internal arcuate fibers	44/5 (H-8)		
Lateral reticulo-spinal tract and dorsal horn	21/2 (A-5)		
Lateral reticulo-spinal tract and ventral horn cells	57/1 (E-1)	36/2 (H-7)	
Medial lemniscus	47/6 (O-2)	31/3 (N-1)	
Medial lemniscus and olivo-cerebellar fibers	36/1 (Q-2)		

TABLE 1—*Continued*

	INSPIRATORY	EXPIRATORY	CONTINUOUS
Medial reticular formation	31/4 (Q-4)	35/2 (P-6)	26/1
			27/4
			28/2
			28/3
			35/6
Medial reticular formation and internal arcuate fibers	14/4 (J-4)	14/4 (J-4)	23/6
	52/1 (Q-3)	25/2 (K-2)	27/7
	52/2 (Q-6)	35/1 (Q-5)	32/1
	54/2 (T-1)		
Medial reticular formation and medial lemniscus			38/1
Medial reticular formation and root fibers of XII			27/5
Medial reticulo-spinal tract	24/1 (N-7)		
Medial reticulo-spinal tract and medial tecto-spinal tract			48/2
Medial tecto-spinal tract	31/1 (P-2)		
	31/1 (N-2)		
Medial tecto-spinal tract and medial lemniscus			27/2
Mesencephalic root of V	51/3		
Nucleus ambiguus	47/4 (M-1)	35/5 (K-1)	
	57/6 (O-8)		
Nucleus ambiguus and lateral reticulo-spinal tract	12/2 (P-7)	14/5 (J-1)	
	28/5 (K-3)	28/5 (K-3)	
	47/1 (L-3)		
	47/2 (L-3)		
Nucleus cuneatus	44/2 (M-5)		23/4
Nucleus gracilis	23/1 (H-3)		
Nucleus of VI			26/2
Nucleus of XI (dorsal efferent)			25/1
Nucleus of XII	24/1 (N-3)	27/3	32/4
	56/4 (M-3)		
or fibers from fasciculus solitarius to	42/1 (L-1)		
Nucleus of XII	24/2 (O-4)	24/2 (O-4)	
root fibers from		25/3 (P-3)	48/1
Olivary nucleus			29/4
Red nucleus	50/5		
Rubro-spinal tract and cerebello-reticular fibers	49/5		
Ventral horn cells	14/2 (D-3)	14/3 (H-2)	
	22/3 (A-4)	48/4 (G-2)	
	44/4 (I-7)	49/2 (I-5)	
	48/5 (G-1)	56/6 (I-6)	
	56/1 (H-1)		

TABLE 1—*Concluded*

	INSPIRATORY	EXPIRATORY	CONTINUOUS
Ventral horn cells and commissuro-cerebellar fibers		18/3 (D-2)	
Ventral horn cells and proprius bundle	15/5 (I-2)		
Ventral reticulo-spinal tract			29/1
Ventral reticulo-spinal tract and fibers of cervical nerve I		44/1 (H-6)	
Ventral reticulo-spinal tract and olivary nucleus	57/4 (O-1)		29/3
Vestibular nucleus			29/2

tion of respiratory potentials, therefore, cannot be determined with the aid of these figures and will be considered later. The figures are of interest in showing the proximity of respiratory potentials to well known nuclear structures in the medulla. Respiratory potentials will be seen to have been encountered most frequently in the region of the obex. After passing a level approximately 5 mm. above the obex they were less frequent and barring occasional exceptions they were absent 10 mm. above the obex, a level corresponding to the cephalic limit of the inferior olivary nucleus and the exiting root fibers of the vagus nerve. This level may temporarily be accepted as the upper limit of the main respiratory mechanism in the medulla.

When we attempt to limit the lower border of this mechanism we meet with a different situation. As might well be suspected, respiratory potentials are found at any level of the cord where respiratory muscles are represented. Our schema, therefore, fails to show a lower envelope of respiratory potentials limiting the lower border of the medullary respiratory mechanism. With the use of table 1, however, we are able to identify the histological source of the potentials and in listing these sources from above downward we reach a level approximately 2 mm. below the obex (in dogs weighing 14 kgm.) where potentials arising in the reticular cells cease. Anatomically this level corresponds to the cephalic limit of the pyramidal decussation, the caudal pole of the lateral reticular nucleus of the medulla oblongata and a point slightly cephalad to the exiting root fibers of the first cervical nerve. This may possibly mark the lower limit of the so-called respiratory center of the medulla.

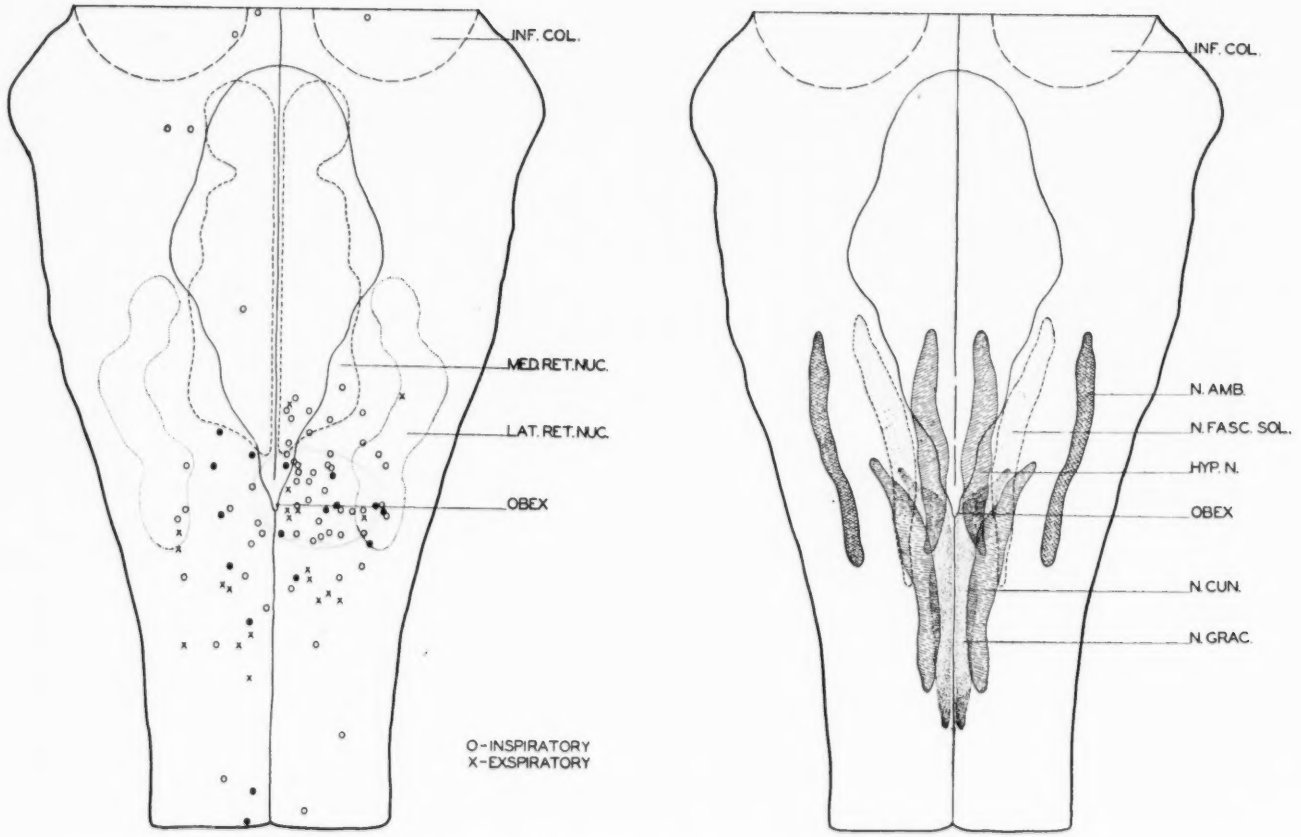
Higher respiratory centers. Many authors have removed the influence of the mid-brain without seriously affecting the mode of breathing. Among these are Legallois (1812), Flourens (1842–51), Longet (1868), Kronecker and Marckwald (1880), Marckwald (1887), Mislawsky (1885), Asher and Luscher (1899), Bazett and Penfield (1922), Lumsden (1923), Spiegel and Enghoff (1925), Schoen (1928), Teregulow (1929), Henderson and Sweet

(1929), Henderson and Craigie (1936). On the other hand a large group have found that removal of all or part of the mid-brain permanently changes respiration. In this group are Martin (1878), Lewandowsky (1881 and 1896), Asher and Luscher (1899), Joukowski (1899), Nicolaides (1905), Trevan (1916), Coombs and Pike (1918), MacLeod (1919), Trevan and Boock (1922), Keller (1928–9), and Coombs (1930). However, if the section is just below the corpora quadrigemina and the vagi are also cut then respiration is invariably affected according to Marckwald (1887), Loewy (1888), Macleod and Page (1922), Lumsden (1923), Trevan and Boock (1922) and Henderson and Sweet (1929). [Nicholson obtains the same effect by cooling in the region of the obex after vagotomy (1936a).] Nonne (1923), Stern (1923), Pette (1923), Lotmar (1926), and Hess and Pollak (1927) from studies of various diseases in which breathing is affected believe that the mid-brain has some regulating control of respiration. Ferrier (1876), Martin (1878), Martin and Booker (1878–79), Christiani (1880), Knoll (1885), Joukowski (1899), Brown (1914) and Coombs (1920) obtained varied effects on breathing upon stimulation at different locations in the mesencephalon.

Transection immediately below the thalamus has no effect upon breathing according to Legallois (1812), Flourens (1842), Longet (1868), Mislawsky (1885), Marckwald (1887), Loewy (1888), Asher and Luscher (1899), Coombs (1918), Trevan and Boock (1922), Schoen (1928), Keller (1929) and Henderson and Sweet (1929). Changes in breathing produced by stimulation of the thalamus have been frequently described (Christiani, 1885; Knoll, 1885; Arnheim, 1894; Joukowski, 1899; Bechterew, 1899; Sachs, 1911; Smith, 1926; Schoen, 1928; and Ransom and Magoun, 1933).

Keller and Hare (1932) produced polypnea by removal of the hypothalamus. Karplus and Kreidel (1909–1910), Asher (1916), Keller and Hare (1932), Ranson and Magoun (1933), Leiter and Grinkler (1934) and Ranson, Kabat, and Magoun (1935), Ectors and Brookens (1936) describe respiratory effects upon stimulation of the hypothalamus.

Considering the accessibility of the respiratory mechanism to almost every variety of impulse—the higher reflex connections of the lower spinal and medullary reflex arcs, the effects of pain, heat, cold, and proprioceptive impulses, and the voluntary and emotional control of breathing—it is only reasonable to expect effects upon breathing from interruption and stimulation of various reflex and fiber tracts. It seemed to us that if there are important mechanisms above the medulla which under all conditions determine the rate and depth of breathing we might be fortunate enough to find these locations and study their function. The region to be explored is considerably greater than that about the obex and, since a complete exploration such as that described for the lower medulla was precluded by the inevitable deterioration of the animal, a staggered exploration was em-



Figs. 1A and 1B. Gross localization of respiratory potentials in relation to nuclear structures.

ployed. Explorations were started at the upper end of the thalamus and soundings were made at 1, 3, 5, 7, 9 and 11 mm. lateral to the mid-line and 2 mm. caudad, at 2, 4, 6, 8 and 10 mm. lateral to the mid-line. After moving the electrodes 2 mm. caudad again, a third series of explorations similar to the first was conducted. In the next animal the initial series of explorations was started, to the best of our measurements, 1 mm. lower or higher than the first explorations of the preceding experiment. In some animals death prevented a complete set of observations; in others, complete soundings were made on one side of the brain. Most of the observations were on the left side.

As in the explorations of the medulla, there was an abundance of miscellaneous potentials but only in five instances did any of them show a respiratory rhythm. These locations are roughly shown in figure 1A. Whether we have employed the proper procedure for locating respiratory potentials in these higher levels remains for future experiments. As was pointed out above an evanescent anesthesia (evipal) was used for these experiments as a precaution against excessive depression at higher levels.

Obviously the findings of those who described no effects on breathing upon transection at the cephalic end of the medulla speak strongly for residence of the principal or pace-setting center in the medulla and at no higher level. The effects of stimulation in the thalamus, hypothalamus and mesencephalon need not necessarily be interpreted in terms of higher "centers." They could be interpreted as indicative of regulative mechanisms which influence the activity of the medullary mechanism. According to Sachs (1911) "There is no center controlling respiration in the optic thalamus.—Any changes in respiration observed on stimulation are reflex effects."

Anatomically there are several theoretical circuits of respiratory impulses which we hoped to encounter (Huber and Crosby, 1929). The scarcity of respiratory potentials may allow several conclusions: During a steady state of anesthesia the upper levels may exert very little regulative action on breathing; some step in our procedure may have led to a reduction or disappearance of higher respiratory potentials; the paths of the impulses may be sufficiently restricted to evade our method of exploration. Despite the fact that twenty experiments were performed we are forced to admit the third possibility through our experience with medullary potentials since in a few of these experiments the most careful exploration failed to reveal respiratory potentials.

Histological localization of respiratory potentials. We have listed potentials in table 1 which have been positively identified with the galvanic lesions placed at the site of these potentials and in figures 8A and B and 9A and B the respiratory potentials are histologically localized with miniature triangles. The potentials are tabulated alphabetically with respect to

possible structures involved and classified in three columns as inspiratory, expiratory, and continuous without respiratory rhythm. The parenthetical notations identify each set of potentials histologically in the cross sectional drawings of figures 8 and 9.

In attempting our electrical study of the respiratory mechanism we were at first satisfied with a gross localization of respiratory potentials which might limit the boundary of the respiratory mechanism, but as the problem grew and the technique improved we were eager to refine the localization of potentials to microscopic or cellular units. It was quite apparent from our observations that the electrodes were very selective. The slightest advance in the penetration produced a readily observable change in the potentials. An up or down movement of 0.1 to 0.2 mm. might call forth an alternate appearance and disappearance of respiratory potentials. However, the limitations of our histological localization are set by the size of lesions we have found advisable to place. Lesions of approximately 0.5 mm. in diameter in many localities are likely to involve more than one group of structures, for example, lesions through a heavy bundle of internal arcuate fibers might possibly involve other structures in the reticular formation. A lesion at the edge of a nucleus might involve a few, but not negligible, units of other nervous elements. The question then arises as to the source of the potentials. Where both cellular and fiber structures are concerned, off-hand preference might be given to cellular source but we would rather hold a guarded position on this point. Though fiber tract potentials are frequently of a weaker order, they may at times record big deflections when neighboring cellular potentials are positively excluded, see for example the centrally placed lesion in the descending root of V (fig. 9A—P8). The conservative identification presented in table 1 seems to be the safer procedure.

The first point to attract attention is the diversity of structures involved in the chain of respiratory impulses. For convenience these potentials may be temporarily grouped under four headings—those obtained in the sensory structures in the medulla and cord, those in the intermediary reticular formations, those in the connecting tracts between the reticular formations and the motor cells of the respiratory muscles, and finally those in the motor cells at the end of the chain. Figures 2A and B and 3A and B show impulses on the sensory side, figures 4A and B and 5 are concerned with the intermediary reticular stations, figures 6A and B with the region of the lateral and medial reticulo-spinal tracts and figures 7A and B with motor stations in the nucleus ambiguus and the ventral horn of the cord. These figures serve only as examples of outstanding phases of the work. The major results have been presented in condensed form in table 1 with very little discussion. Their more complete analysis is left to the reader.

Figure 2A represents a group of incoming sensory signals registered in

the nucleus cuneatus during normal breathing, (for localization of potentials see fig. 9A—lesion M5). They are distinctly discrete, rising in rate during inspiration to 35 per second, and falling abruptly to zero when expiration supervenes. The signals in figure 2B were registered from the nucleus and fasciculus gracilis (fig. 8B—H3). They are from another dog and differ from those of figure 2A in their continuity and lower rate and in

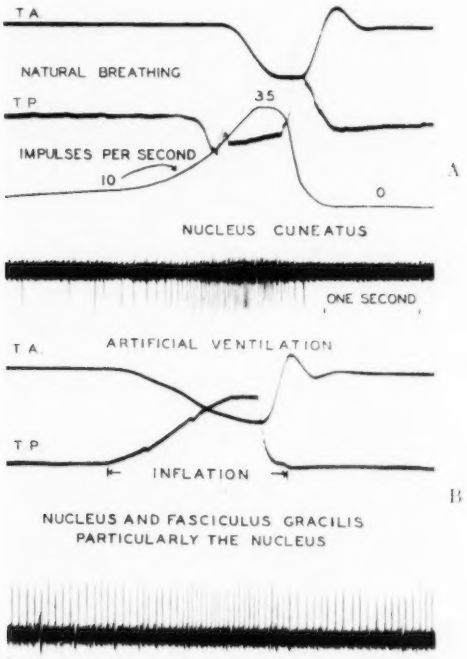


Fig. 2A. Inspiratory signals recorded from the cuneate nucleus during normal breathing.

Fig. 2B. Inspiratory signals recorded from the gracile nucleus during artificial ventilation.

the fact that they occur during artificial respiration administered after complete failure of breathing. The signals are similar to those in figure 2A in that they appear and accelerate during filling of the lungs, suggesting that in both instances they are of proprioceptive origin.

The nucleus fasciculus solitarius acting as the sensory receiving station for vagal impulses might be expected to show a discharge during inspiration when the proprioceptive endings of the lungs are stimulated. Figure 3A

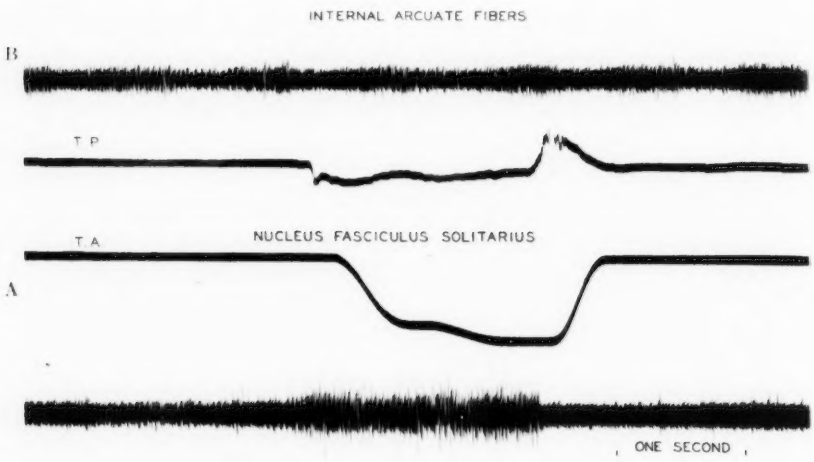


Fig. 3A. Inspiratory signals recorded from the nucleus fasciculus solitarius
Fig. 3B. Inspiratory signals recorded from internal arcuate fibers

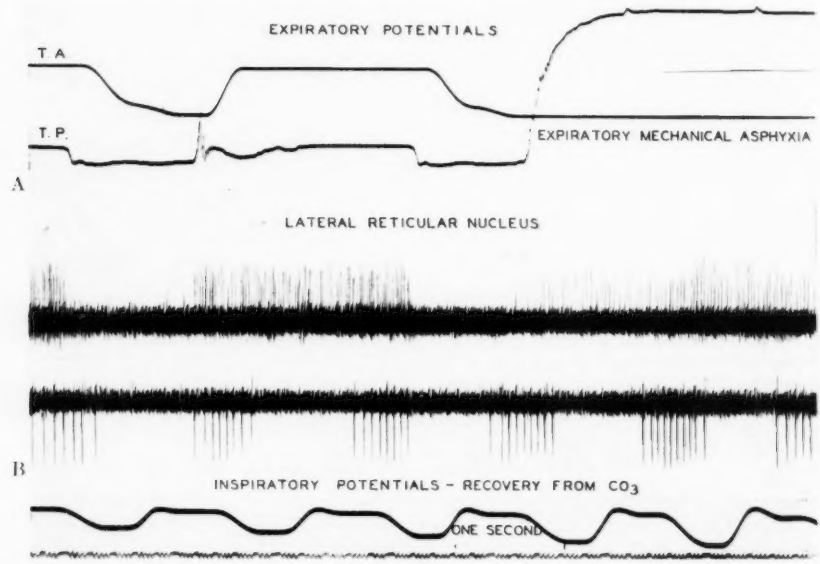


Fig. 4A. Expiratory potentials of the lateral reticular nucleus during normal breathing and expiratory mechanical asphyxia.

Fig. 4B. Inspiratory potentials of the lateral reticular nucleus showing recovery from intravenous injection of sodium carbonate.

shows a volley of signals increasing at the beginning of inspiration and thus agreeing with the linear relation of rate of discharge to lung inflation established by Adrian (1933). But whether the discharge of the nucleus fasciculus solitarius continues to conform with theoretically augmenting incoming signals during further lung extension is only suggested in the heavy discharge. This point is worthy of further study but up to the present we have encountered potentials of the nucleus and fasciculus solitarius only on three occasions despite our large number of medullary soundings.

Another group of sensory signals is indicated in figure 3B where a lesion was placed at the site of inspiratory potentials in the path of a heavy bundle of arcuate fibers (fig. 9A—N5). These potentials conceivably are of proprioceptive origin on their way to higher centers after relay in the nuclei cuneatus and gracilis. They are very indiscrete and in that respect differ from the regular, well defined potentials so commonly found in the reticular gray substance. The recent description of Mingazzini (1928), indicating collateral connections between the cuneate and gracile nuclei and the reticular cells, emphasizes an important stream of signals for the control of breathing.

The reticular formations are undoubtedly in the pathway of respiratory signals. When electrodes are properly placed, that is, in the vicinity of an active unit, the potentials are usually discrete, of large magnitude, and easily recorded. They occur during expiration, as in figure 4A (fig. 8A—T2), and during inspiration, as in figure 4B (fig. 9A—N6). They vary considerably in rate and regularity of discharge. In figure 4b the signals are slower and more regular than in figure 4A. In some instances they are as slow as 30 per minute. They are modified by conditions affecting the respiratory act. If a dog is allowed to inspire, but denied expiration, a reflex slowing of breathing occurs which is accompanied by a prolonged activity of expiratory muscles. Since muscle action potentials persist throughout the expiratory pause, the prolonged firing of the lateral reticular nucleus in figure 4A is worthy of passing comment. So, too, are the effects of intravenous injection of sodium carbonate on the firing of the lateral reticular nucleus in figure 4B which shows recovery from an injection of sodium carbonate. Inspiration at its smallest volume shows 7 slowly repeating discharges per inspiration at the rate of 15 per second and as the tidal air increases the impulses step up to 10 per inspiration and the rate accelerates up to 20 per second. Within nine breaths beyond the limits of the figure the discharge is back to fifteen per inspiration and a rate of 27 per second. The results compare in many respects with those obtained on inspiratory muscles.

A good example of continuous but irregular potentials from a slowly discharging reticular formation (in this instance medial) is illustrated in

figure 5A. The potentials might easily be classified as inspiratory but for the fact that these evanescent accelerations occur in preceding breaths without regard to phase of breathing. Yet, as figure 5B, obtained from the succeeding breath, shows, these potentials are susceptible to modification into an orderly, discontinuous, expiratory rhythm. Inspiratory mechanical asphyxia practically stopped the discharge during the phase of inspira-

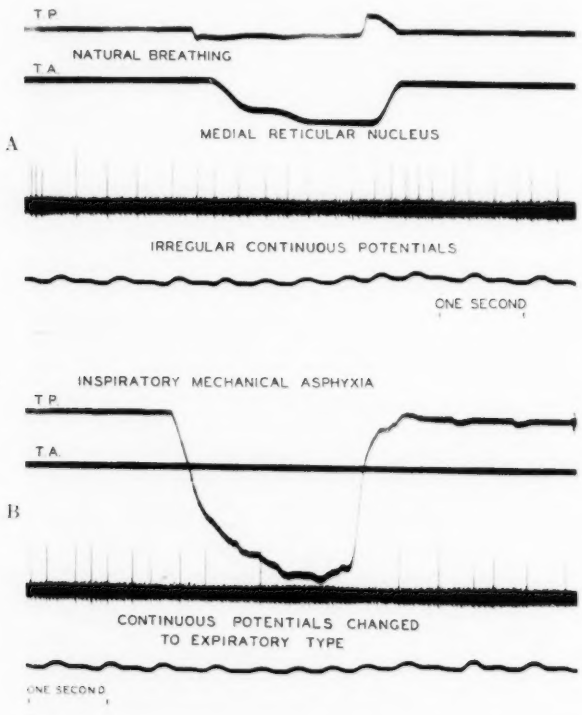
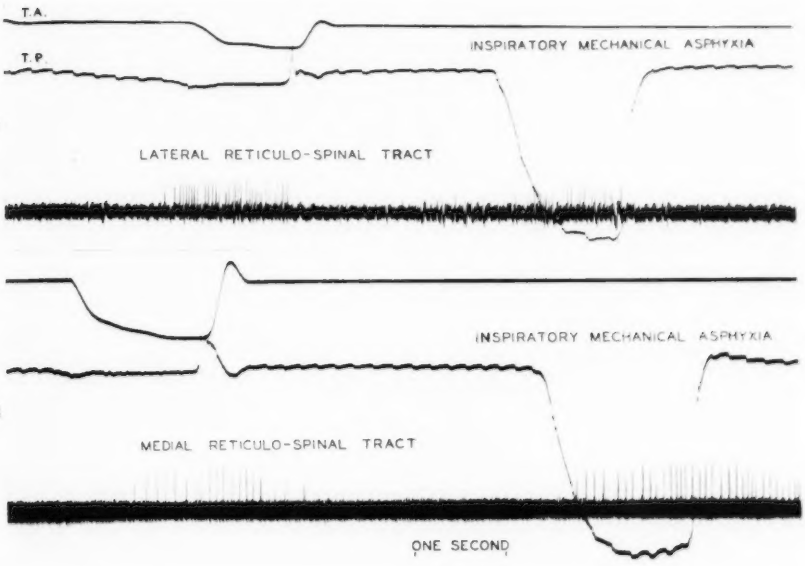


Fig. 5. Continuous potentials of irregular sequence in the medial reticular nucleus changed to the expiratory type by inspiratory mechanical asphyxia.

tion. This state of firing persisted for 7 respiratory cycles and within three breaths after allowing inspiration the potentials were back to their irregular, continuous sequence.

Papez (1926) has described three reticulo-spinal tracts—lateral, medial, and ventral—joining the reticular formations of the medulla, pons and mesencephalon with the cord. These are represented diagrammatically in figure 10. Respiratory signals have been tapped from all of these tracts,

both in the medulla and cord. Inspiratory signals from the medial reticulo-spinal tract are reproduced in figure 6B (9A—N7) and from the lateral reticulo-spinal tract in figure 6A (fig. 9B—3C). The potentials are not always as discrete as shown in these figures but generally resemble those of the reticular formation proper. In figure 6B the inspiratory discharge persists for a long period into the expiratory phase. During inspiratory mechanical asphyxia the potentials are augmented in rate and persist still longer into the expiratory phase. In figure 7B the acceleration during inspiration is less and the augmentation during inspiratory mechani-



Figs. 6A and B. Inspiratory potentials of medial and lateral reticulo-spinal tracts showing effects of inspiratory mechanical asphyxia.

cal asphyxia seems to be missing. This is an unusual response if compared with the responses for an inspiratory muscle (Gesell, 1933).

Respiratory impulses on the efferent side of the arc are shown in figures 7A (fig. 9A—M1) and B (fig. 8B—I2). Potentials from the nucleus ambiguus and from the anterior horn cells are both inspiratory and characteristic for contraction of voluntary muscle (Adrian and Bronk, 1928) and conform with the triangular fusillades of inspiratory muscles described by Gesell (1936B). In the upper record, two motor cells from the nucleus ambiguus are contributing potentials while in the lower record only one ventral horn cell is recording. In the latter record this ventral horn cell

is decreasing in activity and shortly retires completely. Later, mechanical asphyxia recalls it along with neighboring cells into the firing line.

Alae cinereae. Workers in the control of breathing have directed their experiments not only to regional localization but to structural localization as well. Many of their conclusions, as indicated before, have been

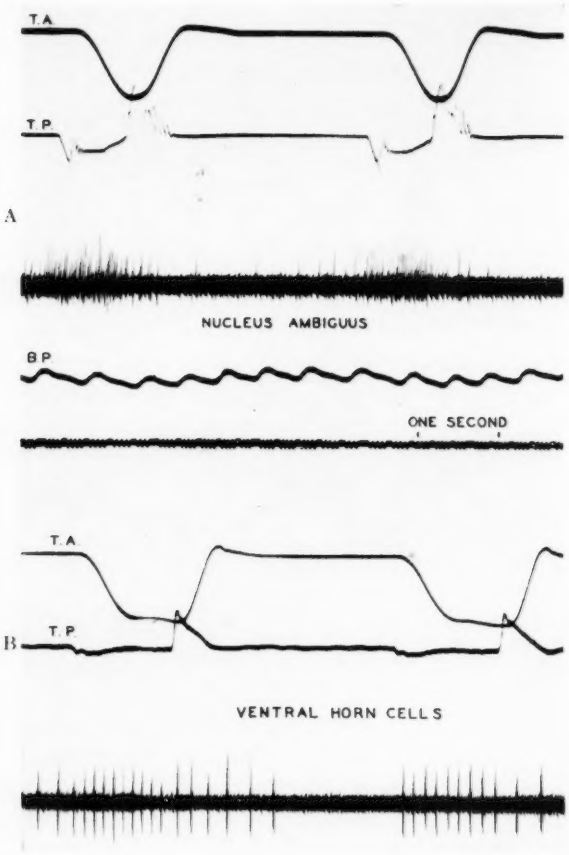


Fig. 7A. Inspiratory signals recorded from N. ambiguus and ventral horn cells

drawn on the basis of effects produced by stimulation or removal of various regions. Our method differs substantially in that any portion of the central nervous system yielding respiratory signals is accepted as part of the complete respiratory mechanism. But only by inference and by confirmation do the findings throw light upon the relative rôle played by the

constituent parts. Attention has centered about the alae cinereae, the fasciculus solitarius and the formatio reticularis.

Schiff (1858) found that he could destroy many parts of the brain stem including Flouren's vital node without stopping respiration. When he destroyed the superior, lateral part of the ala cinerea he caused a cessation of respiration on the corresponding side of the body. Girard (1890) confirmed the results of Schiff. The destruction of the external part of the ala cinerea and the destruction of the posterior part of the nucleus of X on one side causes the cessation of respiration on that side. If the lesion is bilateral then the result is death. This region of the stem has, on the other hand, been destroyed by Gierke (1873) and Gad and Marinescu (1892) without affecting respiration. The finding of respiratory potentials in this region necessarily includes it as part of the respiratory mechanism.

Fasciculus solitarius. Gierke (1873) showed that destruction of the alae cinereae did not stop respiration. He checked the results of his lesions microscopically and came to the conclusion that the only part of the medulla which was essential for respiration was the fasciculus solitarius. This is often known as Gierke's fasciculus. Krause thought that this fasciculus connected the nucleus of the vagus with the phrenic nucleus. Girard (1890) considered the fasciculus solitarius as a descending respiratory path and not as a center. This portion of the brain stem was destroyed by Mislawsky, Gad and Marinescu and Bechterew. More recently Allen (1927) reported that destruction of all the terminal nuclei of the tractus solitarius in the spino-medullary region of the cat and guinea pig caused no permanent disturbance of respiration. Destruction of the trigemino-vagal portion of the nucleus tractus solitarius causes no more permanent change to respiration than severance of both vagi. Here again the finding of respiratory signals in this structure necessarily include it as part of the respiratory mechanism.

Reticular formation. In 1847, Longet destroyed the pyramids and the restiform bodies in the neighborhood of the vagus nucleus, without interfering with breathing, but "the isolated destruction of the 'faisceau intermediare du bulbe', at the same level, caused instantaneous suspension of breathing." He added that this bundle contained numerous nerve cells and gray matter and for that reason appeared to be well suited to serve as a center of some particular function. According to Mislawsky (1885) this appears to be the medial reticular formation.

Since the time of Longet much work has been done on the relation of the reticular formation to respiration. In 1858 Flourens was forced to recognize his respiratory point as a bilateral structure, which was in the dorsal part of the reticular formation, in the neighborhood of the vagal nuclei, on both sides of the raphe just above the obex. Mislawsky (1885) considered the respiratory center as a double nucleus lying in the reticular formation

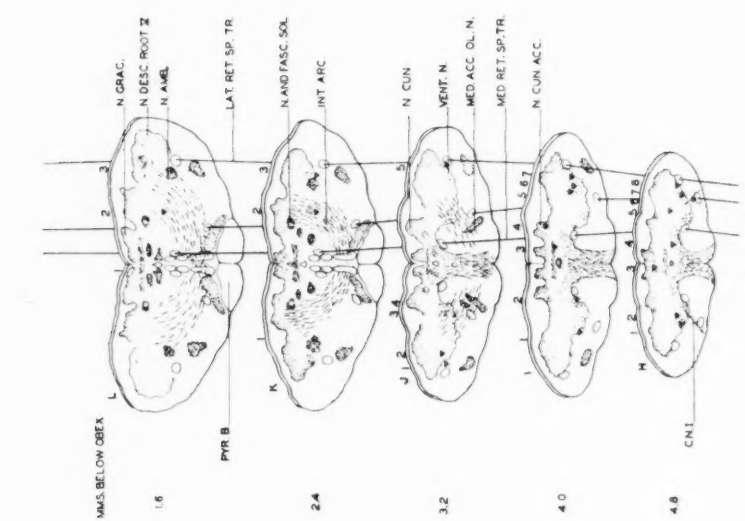

MMS. BELOW OBEX
N. GRAC.
N. DESC. ROOT V
N. AMB.
LAT. RET. SP. TR.
N. AND FASC. SOL.
INT. ARC
N. CUN
VENT. N.
MED. ACC. OL. N.
MED. RET. SP. TR.
N. CUN ACC.
PYR. B
CN I
1.6
2.4
3.2
4.0
4.8

Fig. 8b

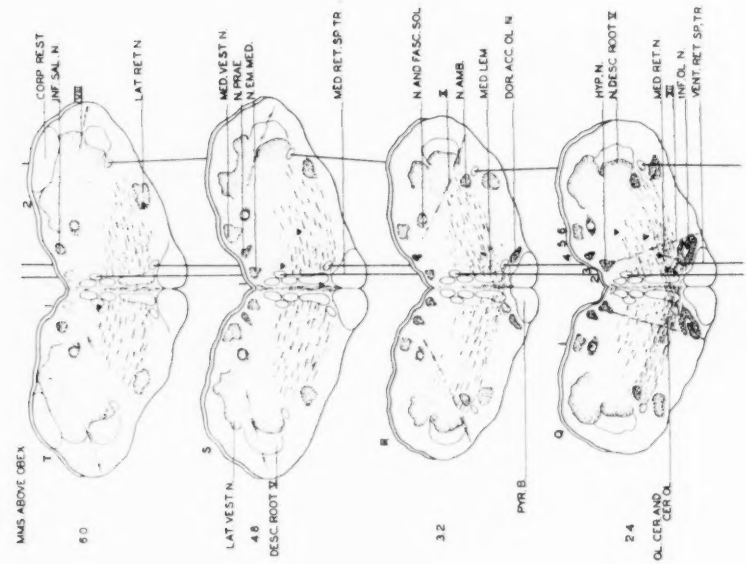

MMS. ABOVE OBEX
CORP. REST.
INF. SAL. N.
LAT. RET. N.
MED. VEST. N.
N. PRAE.
N. EM. MED.
MED. RET. SP. TR.
N. AND FASC. SOL.
N. AMB.
MED. LEM.
DOR. ACC. OL. N.
HYP. N.
N. DESC. ROOT XI
MED. RET. N.
INF. OL. N.
VENT. RET. SP. TR.
LAT. VEST. N.
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Fig. 8a

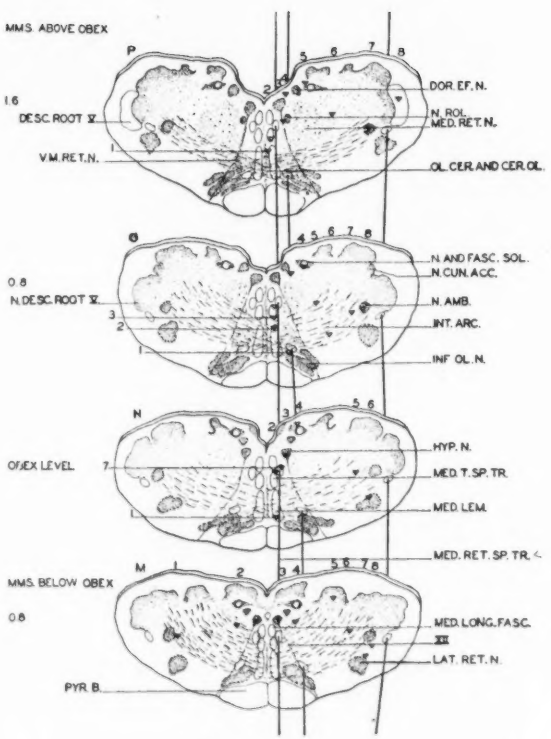

Fig. 9a

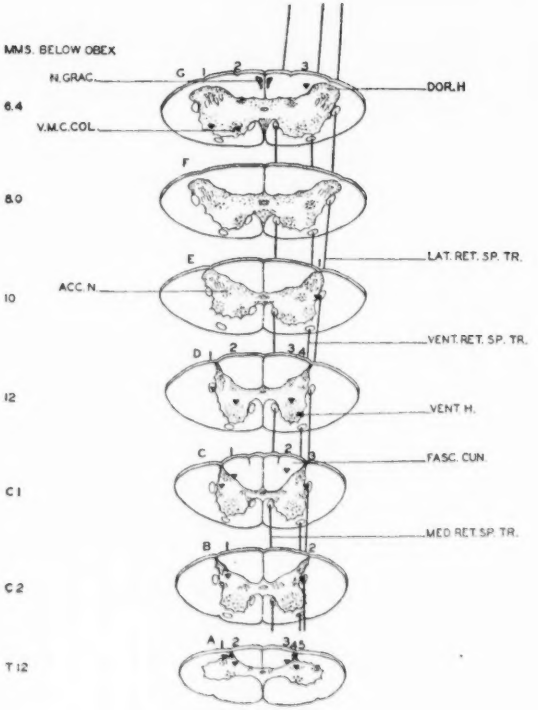

Fig. 9b

Figs. 8 and 9. Histological localization of potentials. Compare with table 1. See key for abbreviations.

on each side of the median raphe mainly internal to the roots of the hypoglossal nerves. This would correspond to the medial reticular formation. Destruction of this area by pricking abolished the respiratory movements immediately while stimulation of the area electrically resulted in a more rapid rate of respiration. Laborde (1890) found a small region on each side of the raphe and just above the point of the calamus which when destroyed for half the thickness of the medulla permanently stopped respiration. This perhaps corresponds to the medial reticular formation. He obtained no results from destruction of parts of the brain other than the medulla. Gad and Marinescu (1892) destroyed various parts of the brain stem with hot glass rods. They showed that only those lesions which were deep in the reticular formation stopped respiration. They could destroy Flouren's vital node, the external part of the alae cinereae and the solitary bundle without stopping respiration (regions of Flourens, Longet, Schiff, Gierke, and Mislawsky). These same workers stimulated deep in the stem with fine needles varnished except at the tips. When these needles were in the reticular formation there was an acceleration of respiratory rate and not a tetanization of the inspiratory muscles as follows stimulation of the lateral bundles of the cervical cord. In 1894 Arnheim found that stimulation between the nucleus of VI and the calamus accelerated respiration if the electrodes touched the reticular gray. Stimulation of the motor nucleus of VII caused only the muscles of the nose to respond, contrary to the opinion of Grossmann. Kohnstamm (1900) considered the reticular formation as an important regulatory mechanism for the nuclei supplying respiratory muscles. After section of the spinal cord in its upper part he obtained a degeneration of the reticular formation in the region Bechterew describes as his center. Bechterew (1908) states "The respiratory nucleus lies, as my own investigations show, only for the lesser part medial to the hypoglossal roots, for the greater part lateral to them. Each time I obtained, on transection or circumscribed destruction of the medulla oblongata, an arrest of respiration, in those cases when that small region of gray substance, which lies just above the calamus scriptorius chiefly lateral to the hypoglossal nucleus in the depths of the medulla oblongata, was destroyed." In 1931 Finley reported upon two cases of respiratory failure. His autopsies show congestion of the upper cervical cord, with round cell infiltration and partial destruction of the anterior horn cells and bilateral areas of necrosis in the reticular formation. From his review of the literature and from these two cases of respiratory failure he concludes that one of the main integrative levels for respiratory impulses lies in the reticular formation. Johnston (1932) found marked damage to the cells of the medullary and pontal centers in six cases of death from pneumonia. The sensory nuclei showed more damage than the motor nuclei. Cells of the reticular gray matter showed the most severe degenerative changes.

Keller (1928–29) from his studies of the polypnea mechanism concludes that the reticular formation is not important in the transmission of the impulses since extensive lesions of various kinds can be made in the medial part of the medulla and pons without affecting rapid respiration while lesions in the lateral regions stop the rapid respiration. According to Papez (1926) and Allen (1927), the reticulo-spinal tracts are employed to conduct the impulses to the motor cells of the respiratory muscles. According to Papez, the medial reticulo-spinal tract is the most extensive. It has its origin from large reticular cells of the pons and isthmus and from large cells of the upper medulla oblongata. The majority of the fibers of this tract remain on the same side, but a few cross and descend with fibers of the opposite side. The lateral reticulo-spinal tract has its origin from pontal reticular cells medial to the masticator nucleus, and from reticular cells near the mid-line extending to the genu of the facial nerve. The ventral reticulo-spinal tract in some instances appears to be a direct continuation of the descending limb of the brachium conjunctivum coming possibly from the nuclear masses around the brachium conjunctivum, but not all lesions in this region yield the tract. Other evidence indicates that it is crossed, decussating through Bechterew's reticular nucleus and having its origin in the ventro-lateral tegmental nucleus just lateral to the medial longitudinal bundle. It then follows along the dorsal accessory olive into the ventral funiculus of the cord.

Experimental findings indicating that the reticular formation serves as the main station for integration of the respiratory signals are amply confirmed by our electrical soundings. No other structures yielded respiratory potentials in such abundance or with such certainty and the tapping of potentials from the reticulo-spinal tract is further strong evidence of the integrating rôle of the reticular system. The frequent occurrence of potentials in the lateral reticular nuclei marks a more lateral extension of the respiratory mechanism in the formatio reticularis than that recently described by Henderson and Craigie (1936) if our interpretation of their measurements is correct. Through localized sectioning of fiber paths, Allen concludes that the ventral and lateral reticulo-spinal tracts are the main channels conducting impulses from the cerebrum and superior colliculus to the respiratory nuclei of the spinal cord. Our findings confirm this view and add the medial reticulo-spinal tract as an accessory highway. Furthermore, our findings indicate that the normal pathways of respiratory impulses are similar to those of modified breathing produced by stimulation of higher centers (Allen 1927).

Of no little interest are the five sets of potentials encountered in our total of twenty experiments on higher "respiratory centers." Of the five sources of potentials four were localized and one lesion was lost (see the upper four locations in fig. 1A). One lesion involved the medial reticulo-spinal formation, internal arcuate fibers, and possibly the medial reticulo-spinal

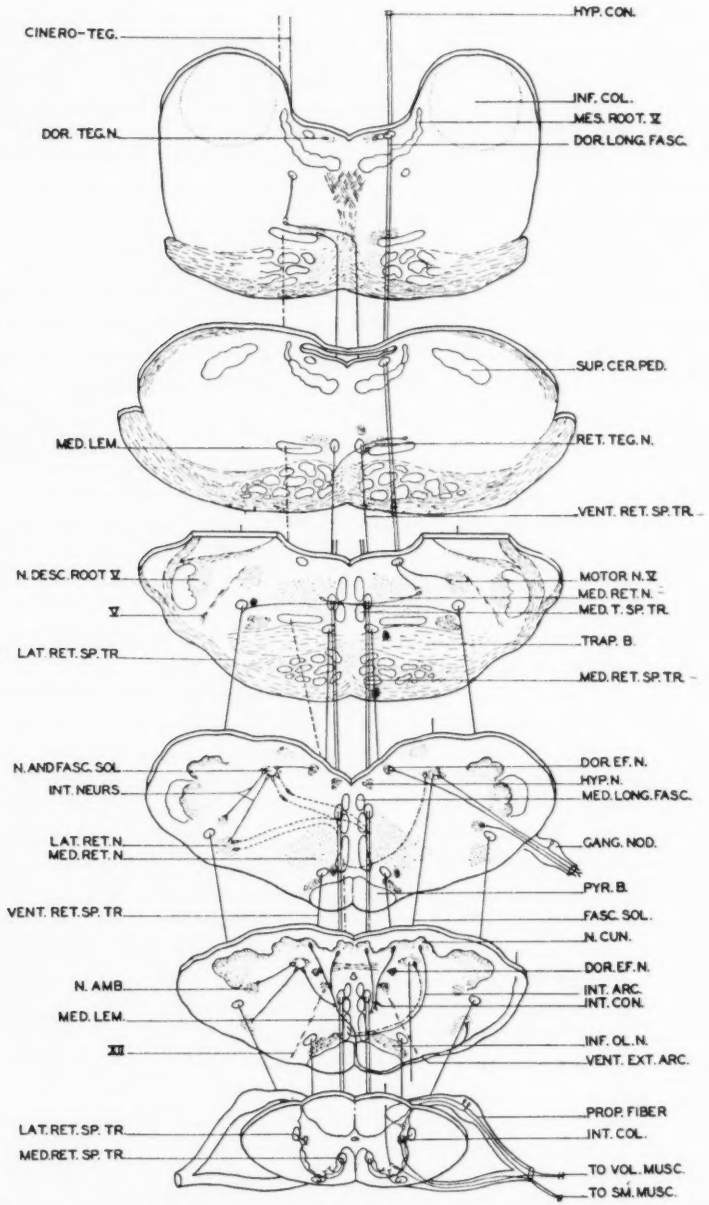

Fig. 10. Schema of central respiratory mechanism. See key for abbreviation.

tract. The remaining three involved the brachium conjunctivum. Thus they suggest a contributing control through the cerebellum despite our failure to find respiratory signals in a preliminary exploration of that structure, and furthermore, they support Papez's suggestion regarding the brachium conjunctivum as a source of fibers in the ventral reticulospinal tract.

Discussion. Bearing in mind the destructive effects of pin prick lesions, such as described by Flourens, we were surprisingly encouraged in the possibilities of our procedure during our early experiments on the vital region of the medulla. Dense riddling of the entire lower medulla in some animals seemed to have no effects whatever on breathing and permitted extensive observations. To be sure, the animals deteriorated, some very slowly and others rapidly, but previous decortication seemed more related to collapse than did sounding for potentials. The fineness of our electrodes could easily account for the lack of damage but repeated galvanic lesions seemed to produce only momentary effects while the current was flowing. This immunity to damage is probably related to the structural and func-

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Acc. N.—Spinal accessory nucleus; *Cinero-Teg.*—Cinero-tegmental connection; *C. N. I.*—Cervical nerve I; *Corp. Rest.*—Corpus restiforme; *Desc. Root V*—Descending root of trigeminal; *Dor. Acc. Ol. N.*—Dorsal accessory olivary nucleus; *Dor. Ef. N.*—Dorsal efferent nucleus; *Dor. H.*—Dorsal horn; *Dor. Long. Fasc.*—Dorsal longitudinal fasciculus; *Dor. Teg. N.*—Dorsal tegmental nucleus; *Fasc. Cun.*—Fasciculus cuneatus; *Fasc. Sol.*—Fasciculus solitarius; *Gang. Nod.*—Ganglion nodosum; *Hyp. Con.*—Hypothalamic connection; *Hyp. N.*—Hypothalamic nucleus; *Inf. Col.*—Inferior colliculus; *Inf. Ol. N.*—Inferior olivary nucleus; *Inf. Sal. N.*—Inferior salivary nucleus; *Int. Arc.*—Internal arcuate fibers; *Int. Con.*—Internuncial connections between gracile and cuneate nuclei and medial reticular formation; *Int. Col.*—Intermediolateral cell column; *Int. Neurs.*—Internuncial neurones; *Lat. Ret. N.*—Lateral reticular nucleus of the medulla oblongata; *Lat. Ret. Sp. Tr.*—Lateral reticulo-spinal tract; *Lat. Vest. N.*—Lateral vestibular nucleus; *Med. Acc. Ol. N.*—Medial accessory olivary nucleus; *Med. Lem.*—Medial lemniscus; *Med. Long. Fasc.*—Medial longitudinal fasciculus; *Med. Ret. N.*—Medial reticular nucleus of the medulla oblongata; *Med. Ret. Sp. Tr.*—Medial reticulo-spinal tract; *Med. T. Sp. Tr.*—Medial tecto-spinal tract; *Med. Vest. N.*—Medial vestibular nucleus; *Mes. Root V*—Mesencephalic root of trigeminal nerve; *Motor N. V.*—Motor nucleus of trigeminal nerve; *N. Amb.*—Nucleus ambiguus; *N. and Fasc. Sol.*—Nucleus and fasciculus solitarius; *N. Fasc. Sol.*—Nucleus fasciculus solitarius; *N. Cun.*—Nucleus cuneatus; *N. Cun. Acc.*—Nucleus cuneatus accessorius; *N. Desc. Root V*—Nucleus descending root of trigeminal nerve; *N. Em. Med.*—Nucleus emminentia medialis; *N. Grac.*—Nucleus gracilis; *N. Prae.*—Nucleus praeposticus; *N. Rol.*—Nucleus of Roller; *Ol. Cer. and Cer. Ol.*—Olivo-cerebellar and cerebello-olivary fibers; *Prop. Fiber*—Proprioceptive fiber; *Pyr. B.*—Pyramidal bundle; *Ret. Teg. N.*—Reticular tegmental nucleus of Bechterew; *Sm. Musc.*—Smooth, involuntary muscle; *Sup. Cer. Ped.*—Superior cerebellar peduncle; *Trap. B.*—Trapezoid body; *Vent. Ext. Arc.*—Ventral external arcuate fibers; *Vent. H.*—Ventral horn; *Vent. N.*—Ventro-lateral nucleus; *Vent. Ret. Sp. Tr.*—Ventral reticulo-spinal tract; *V. M. C. Col.*—Ventro-medial cell column; *V. M. Ret. N.*—Ventro-medial reticular nucleus; *Vol. Musc.*—Striated, voluntary muscle.

tional organization of the reticular formation. The impression we have gained is that the potentials are relatively few and scattered and that the anatomical description "scattered reticular gray matter" is descriptive of its function as well. The relative scarcity of reticular potentials, the large size and spacing of the cells, considered with the copious innervation of muscles as represented in the ventral column of the cord suggest a multiple innervation of the ventral horn cells with a high step-up ratio.

The fact that potentials vary considerably in rate, and that they may vanish and reappear under conditions modifying the respiratory act, indicates that the mechanisms of recruitment and of adjustment of rhythm are at the disposal of the reticular formation. We would venture to say, however, that the acceleration of reticular firing during inspiration is not equal to that of the motor nerve cells.

The higher incidence of inspiratory potentials compared with expiratory (3 to 1 ratio) agrees with the greater incidence of contraction of inspiratory muscles noted in systematic electric exploration of respiratory muscles (Gesell, 1936a).

The absence of a systematic or localized arrangement of inspiratory and expiratory potentials, as plotted in figure 1A, indicates a lack of grouping of cells into inspiratory and expiratory nuclei and changing potentials with progressive penetration of the electrodes yields the same conclusion. This point is illustrated by an example of a single sounding of 3 mm. extent, which successively tapped-off seven sets of potentials in the order given—inspiratory, expiratory, expiratory (new set), regular frequency without respiratory rhythm, expiratory, inspiratory, and expiratory. The previous conceptions of compact, definitely localized inspiratory and expiratory centers, held by some investigators, probably should be revised in the light of our findings. We have no evidence of special groupings of potentials favoring Lumsden's views.

The great abundance of potentials of continuing regular frequency was a uniform finding and has raised the question of the source of the drive perpetuating these signals. We have seen an instance in which such signals were modified to take on a respiratory rhythm (fig. 5B), but have no evidence to ascertain the destination of these signals. They may be signals on their way to striated muscle or to the involuntary muscle of heart, blood vessels or lungs. Their relatively low source in the medulla probably favors the first view. Granting that, it is reasonable, in a steady state of anesthesia, to expect a steady reflex drive through the proprioceptive and cerebellar route for muscles in fixed position. Should any of these muscles be called to contribute towards pulmonary ventilation (as in fig. 5) steady fire would give way to interruption corresponding to respiratory rhythm.

An attempt to construct a schema of the central respiratory mechanism in our state of knowledge is an invitation to error and incompleteness but a few tentative reflections may not be amiss. It would seem: that the

integrative function of the reticular formation for respiratory movements is more definitely established by our findings; that we are privileged to look upon this formation as the part about which the incoming and outgoing respiratory highways are constructed; that the reticular gray matter is influenced by every type of sensory signal, but most importantly through the fifth, ninth, tenth, eleventh, and twelfth cranial nerves and the sensory roots of the cord; that signals entering by these channels may reach the reticular gray by various paths (see schema) some direct, others by circuitous routes; that removal of the reticular gray matter would remove all adequate means of integrating respiratory signals and leave no organization for concerted orderly recruitment of respiratory muscles and therefore lead to a respiratory death; that the reticular gray directs its signals in orderly fashion down the reticulo-spinal tracts at the end of which the respiratory motor neurones are fired; that a mechanism capable of further control may exist at this point of firing.

Our experiments offer no entering wedge to open the question of automaticity of reticular gray matter.

In conclusion we feel that our present experiments are of a preliminary nature, that they offer mainly a skeleton of the respiratory mechanism, on which we hope to build a more complete picture of respiratory signals under conditions modifying the respiratory act.

SUMMARY AND CONCLUSIONS

The structure of the central mechanism controlling breathing and the function of its constituent parts were studied with a method of electrical sounding. The central gray axis stem of the dog including the diencephalon, mesencephalon, pons, medulla, and upper cervical cord were systematically explored with fine needle electrodes bared at the tips. Potentials showing a respiratory rhythm were photographed and their sources histologically localized.

Respiratory potentials were found in greatest abundance and with greatest certainty in the region about the obex. The intimate relation of these potentials to important respiratory nuclei was graphically presented.

Explorations of the mesencephalon and diencephalon in twenty experiments revealed only five sets of respiratory potentials, indicating that the main "center" or controlling mechanism resides in the medulla oblongata and that impulses coming from above are primarily of a regulative nature.

Exploration of the cord showed extensive respiratory potentials. This finding conforms with the necessary chain of impulses for the innervation of respiratory muscles.

The upper limit of the medullary respiratory potentials cuts across the upper quarter of the N. hypoglossus, N. ambiguus, and N. fasciculus solitarius. The incidence of respiratory potentials extending continuously

from the medulla through the cord prevents similar establishment of the lower border of the medullary respiratory mechanism, but a line marking the disappearance of reticular formation potentials places the lower border at the cephalic edge of the pyramidal decussation. These limits are in agreement with findings of many workers localizing the respiratory center in the medulla oblongata.

Lesions at the sites of respiratory potentials involved a large variety of structures indicating the diversity of highways for respiratory signals. These structures and their associated potentials are tabulated in detail.

The most common source of respiratory signals was in the reticular formation. It is concluded that these formations are intermediary stations interposed between incoming and outgoing signals and that they are essential to the respiratory act.

The incidence of inspiratory potentials, like that in respiratory muscles, was considerably greater than the incidence of expiratory potentials.

There was no anatomical grouping of signals indicating a structural collection of cells as demanded by previously postulated compact inspiratory and expiratory centers.

The occurrence of potentials in the brachium conjunctivum suggests a contribution of the cerebellum to the integration of the respiratory act.

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THE EFFECT OF INTRAVENOUS ADMINISTRATION OF PROTAMINE INSULIN

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During some clinical investigations of the effects of protamine insulin compound (protamine insulin, Lilly[1]) which are now being carried out under the direction of Dr. James J. Waring, it seemed desirable to investigate some of the properties of the compound on experimental animals in which the results would not be complicated by the presence of disease. Hagedorn, Jensen, Krarup and Wodstrup (1) first reported the development of protamine insulinate. They found that the material was absorbed from the site of injection more slowly than ordinary insulin, a fact which has been confirmed by direct observations on rabbits by Beecher and Krogh (2). Slower absorption results in a prolongation of activity which makes it possible to treat patients with smaller doses of insulin. Furthermore, the diurnal fluctuations in the blood sugar become less pronounced and thereby are made to assume a condition more nearly like that of the normal individual. These latter findings have been confirmed by Root, White, Marble and Stotz (3), by Sprague, Blum, Osterberg, Kepler and Wilder (4), and by Kerr, Best, Campbell and Fletcher (5).

METHODS. The experiments were carried out upon female albino rabbits. Females were chosen because of the report of Dotti (6) that the female is more constant than the male in its response to insulin. The weights of the animals ranged from 1500 to 2400 grams. No special diets were used; the rabbits were maintained on the stock colony diet. Each animal was subjected to a 24 hour fast before administration of the insulin or protamine insulin. The dose used was 1.5 unit per kilogram of body weight.

Blood was withdrawn from the ear vein before administration of the insulin or protamine insulin and at ½, 1, 2 and 3 hours thereafter. Blood sugar was determined by the method of Folin and Wu (7) modified for use with the Peebles-Lewis colorimeter (8). This method gives values for the total reducing substance of the blood.

[1] The protamine insulin compound was furnished by Eli Lilly & Co., Indianapolis, whose coöperation we gratefully acknowledge.

TABLE 1

Comparison of the effects of insulin and protamine insulin after intravenous administration

RABBIT NUMBER	BLOOD SUGAR, MGM. PER 100 CC.				
	Before insulin	½ hour	1 hour	2 hours	3 hours
Protamine insulin					
1	*112*	*72*	*60*	*76*	*98*
2	*108*	*67*	*63*	*90*	*116*
3	*Lost*	*78*	*55*	*70*	*114*
4	*106*	*60*	*28*	*60*	*94*
5	*98*	*60*	*47*	*64*	*80*
6	*116*	*62*	*60*	*72*	*96*
7	*104*	*59*	*31*	*69*	*98*
Protamine insulin and regular insulin*					
8	*114*	*72*	*43*	*40*	*60*
	105	57	32	38	76
9	*88*	*78*	*50*	*88*	*106*
	109	88	56	92	113
10	*90*	*68*	*47*	*53*	*88*
	90	55	39	49	68
11	*92*	*60*	*44*	*58*	*89*
	120	58	45	77	120
12	*94*	*62*	*48*	*56*	*65*
	112	50	44	53	98
13	*78*	*63*	*51*	*66*	*86*
	109	54	55	72	99
14	*130*	*68*	*43*	*67*	*102*
	118	47	40	84	120
15	*110*	*91*	*45*	*57*	*104*
	119	89	57	76	120
16	*100*	*67*	*40*	*60*	*90*
	118	66	48	60	98

* Protamine insulin in italics.

Nine animals were given protamine insulin subcutaneously. Sixteen additional animals received protamine insulin intravenously. To nine of these latter sixteen animals regular insulin was given intravenously in

order that we might compare the results. The animals received protamine insulin first and, after a rest period of at least five days, they were given the same dose of regular insulin.

RESULTS. In confirmation of the results obtained with diabetic patients, our results with rabbits show that after subcutaneous injection the effect of protamine insulin is greatly prolonged as compared to the response of the blood sugar to regular insulin. Some of the results in which there was a fairly rapid action together with a prolongation of the effect suggested to us that we might have inadvertently injected some of the material into a small blood vessel as well as under the skin. We carried out experiments to determine the effect of intravenous administration of protamine insulin, and the results of these experiments are recorded in table 1. The results show that there is no essential difference between the reaction of the same animal to the insulin and protamine insulin when given intravenously. The low solubility of protamine insulin at the pH of the body appears to affect the activity of the compound only so far as its absorption from a subcutaneous injection is concerned. When it is directly dispersed in the blood, it acts in a manner practically identical with that of ordinary insulin.

The failure to obtain delayed reaction after the intravenous administration of protamine insulin is further evidence that the prolonged activity of the compound which follows subcutaneous injection depends upon its delayed absorption. Protamine insulin is a particulate suspension which is least soluble at pH 7.3 (1). When this type of substance is administered subcutaneously it is deposited in a restricted area in the tissue spaces, and it has available only a small amount of slowly moving solvent fluid. This probably accounts for the fact that its absorption into the blood stream is slow and difficult. However, when it is injected into the blood it is rapidly distributed into a large amount of solvent. Under these latter circumstances the factor of low solubility at the existing pH does not retard solution of the compound to the point where there is an appreciable delay in its action. For this reason the blood sugar responds promptly to the intravenous administration of protamine insulin.

SUMMARY

The prolonged effect of protamine insulin after subcutaneous injection has been confirmed. The action of the compound after intravenous injection, however, does not differ significantly from the action of regular insulin similarly injected.

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THE MECHANISM OF THE INHIBITORY ACTION OF VASODILATOR NERVES

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The regulation of the tone of blood vessels is to a large extent effected by two antagonistic kinds of nerve fibers, vasoconstrictor and vasodilator. Impulses discharged through sympathetic nerves produce contraction of the muscular coat of the vessels and keep them normally in a condition of moderate constriction. Dilatation, on the other hand, which is due to a lengthening of the circular muscle fibers, can in the intact animal result from a cessation or diminution of vasoconstrictor activity. There is, however, a second mechanism for producing vasodilatation, and that is the action of specific inhibitory nerves. The latter mechanism, which alone is of interest in this work, is one of the most important cases of peripheral inhibition.

At least two hypotheses have been offered to explain this phenomenon. It has been suggested that the stimulation of inhibitory fibers produces a depression of the contractile mechanism. Other authors have assumed that the inhibition is an active response by means of which the muscle fibers can be set at a new and greater length. However, little experimental work has been done to verify these hypotheses. In the experiments which are reported here I have attempted to study this problem by influencing the muscular coat of blood vessels simultaneously by excitatory and inhibitory impulses and by a quantitative study of the interaction of their effects.

MATERIAL AND METHOD. In order to avoid the complications of a normal circulation it seemed advisable to use a perfusion method. The reactions of the blood vessels of a perfused preparation can be conveniently studied by recording the rate of flow of the perfusion fluid. It is well known that the largest part of the resistance to flow is located in the arterial system, so that changes in the rate of flow indicate with fair accuracy the changes in width of the peripheral vessels, mainly those of the arterioles, of the perfused organ.

The hind leg of the frog proved to be very suitable for these experiments. The rate of flow of the perfusion fluid going into the preparation

was measured by a sensitive flowmeter. Constriction of the blood vessels was produced by stimulating the sympathetic trunk; purely inhibitory effects were obtained by stimulating the dorsal roots.

The flowmeter which was used in these experiments is based on the principle of the Pitot tube (fig. 1). The perfusion fluid (Ringer's solution containing 0.65 per cent NaCl, 0.02 per cent KCl and 0.015 per cent $CaCl_2$ adjusted to pH 7.5 by phosphate buffer) passes through a capillary, *C*, the pressure difference across which is recorded by the differential manometer, *DM*. This manometer consists of two thin rubber membranes about 4 mm. in diameter connected together by a piece of metal, *X*, whose movements are transmitted by the torsion spring, *T*, and are recorded by a small mirror. The deflection of the meter is accurately

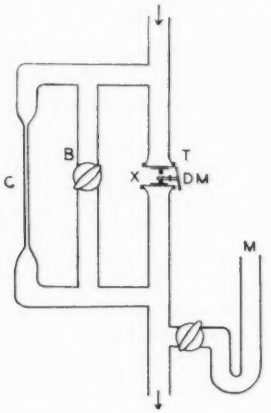

Fig. 1. Diagram of flow meter. *C*, capillary tube; *B*, bypass; *DM*, differential manometer; *M*, U-tube manometer; *T*, torsion spring; *X*, metal piece connecting the two rubber membranes.

proportional to the rate of flow. In the experiments which will be reported 1 mm. deflection on the recording camera corresponded to a change of flow of about 0.25 cmm. per second; this is a change of 1 to 2 per cent of the maximal rate of flow through the preparation.[1]

[1] It should be noted that the pressure difference recorded by the differential manometer is only accurately proportional to the rate of flow at constant velocity. If the diameter of the vessels diminishes rapidly, a pressure will be produced by this contraction. A new stationary state will be established after some time which depends on the resistance to flow on both sides of the constricted vessel. The question whether this and the lag of the flowmeter introduces an appreciable distortion of the records of the contractions can be empirically tested by rapidly compressing the limb. It was found that a new constant rate of flow is established in a fraction of a second. The lag of the recording system is therefore small compared with the time relations of the contractions of the blood vessels.

The manometer, *M*, which is disconnected while the flow is recorded, measures the perfusion pressure; it was 10 to 12 cm. water in the experiments. For greatest sensitivity this pressure should be about one-half of the total pressure difference between the bottle containing the perfusion fluid and the preparation. This can be adjusted by choosing a capillary of suitable width. For most purposes the sensitivity so obtained is too great and wider capillaries can be used.

Drugs are applied by injection into the rubber connection near the preparation. During the injection the manometer, *M*, is connected to the flowmeter. This provides an outlet for the fluid and prevents the production of an excessive pressure by the injection.

Only Rana pipiens proved to be suitable for the experiments. The peculiar observation was made that in bullfrogs (Rana catesbiana) stimulation of the dorsal roots regularly produced vasoconstriction if the vessels were dilated, dilatation if the vessels were strongly constricted. This is presumably due to the presence of sympathetic fibers in the dorsal roots in this species. This observation is in line with older findings of the occasional occurrence in frogs of dorsal root fibers supplying the urinary bladder (Langley and Orbeli, 1910, review on this question: Brücke, 1927). In Rana pipiens, on the other hand, slight constrictor effects were observed only rarely on stimulation of the dorsal roots.

The animals were narcotised with ether, decapitated and eviscerated. The aorta was preserved and the cannula inserted into one of the femoral arteries so that only one leg was perfused. The sympathetic trunk of the perfused side was dissected out, leaving the rami to the sciatic nerve intact. One pair of stimulating electrodes was placed on the sympathetic trunk as close to the sciatic plexus as is possible without spread of the stimulating current. For the preparation of the dorsal roots the spinal canal was opened ventrally and all nerves except the dorsal roots supplying the sciatic nerve of the perfused leg were cut. The stimuli were usually applied to the whole caudal part of the spinal cord, although in some cases the cord was removed and the stimulating electrodes were attached to the roots directly. The nerves were stimulated by condenser discharges. A maximal stimulus was obtained by a condenser of 0.3μ F and 6 to 8 volts. To minimize polarization at the electrodes during the longer periods of stimulation a commutator was used to reverse the direction of alternate shocks. The dorsal roots were always stimulated at a frequency of about 20 shocks per second, the sympathetic fibers at various rates.

Results. Figure 2A is an example of the records obtained with the method just described. A constriction of the blood vessel muscles was produced by stimulating the sympathetic trunk for 15 seconds. The record obtained with the flowmeter is similar to a record of a smooth

muscle contraction obtained with the conventional recording methods. The rapid diminution of flow corresponds to the rising phase of the contraction; the gradual return to the previous level represents the relaxation phase.

General effects of dorsal root stimulation. Immediately after the perfusion is started the blood vessels are completely dilated as shown by the fact that neither dorsal root stimulation nor acetylcholine produces any change of flow. After 1 to 2 hours however the vessels always begin to constrict without any external stimulus. Repetitive stimulation of the dorsal roots then produces, after a latent period of about 3 seconds, a maximal dilatation (fig. 3A). This lasts for about one minute after which the vessels again constrict slowly even though the stimulation of the dorsal roots is continued. After an intermission of a few seconds the

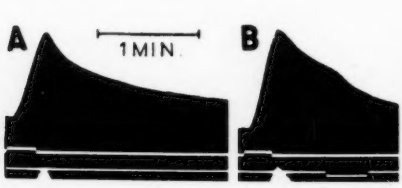

Fig. 2

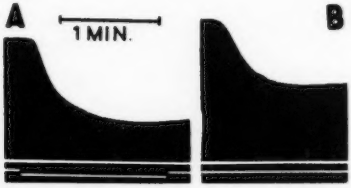

Fig. 3

Fig. 2, A and B. Two contractions of the muscles of blood vessels produced by sympathetic stimulation. During the relaxation of the second contraction, B, the dorsal roots were stimulated. Upper signal marks stimulation of the sympathetic, lower signal that of the dorsal roots.

Fig. 3, A. and B. Dilatation of the blood vessels of the perfused frog leg, A, on stimulation of the dorsal roots (indicated by the lower of the two signals). B. Application of acetyl-β-methylcholine, 1:2,000,000. The initial constriction of the blood vessels developed spontaneously and was maintained without external stimuli.

stimulation is fully effective again. Another effect of dorsal root stimulation is its influence on the speed of relaxation. Following a strong contraction the relaxation is very slow, but is considerably accelerated by stimulation of the dorsal roots (fig. 2B).

An effect similar to that of dorsal root stimulation can be obtained by injecting acetylcholine into the perfusion fluid (fig. 3B). Following such an injection there is a marked dilatation. Likewise acceleration of the relaxation following strong sympathetic stimulation can be produced by acetylcholine.

Dilatation of blood vessels due to dorsal root stimulation has already been demonstrated in frogs by Pearce (1913) and Doi (1924). Whereas in mammals this reaction occurs mainly in the skin, Krogh, Harrop and Rehberg (1922) showed that in the frog the vessels in skeletal muscles participate to an appreciable extent. My own observations are in agree-

ment with this and further indicate that all the vessels of the perfused leg are affected by the dorsal root fibers. This is shown by the fact that the maximal dilatation produced by dorsal root stimulation is usually as great or nearly as great as that produced by acetylcholine. Removal of the skin left the effect of the dorsal root stimulation practically undiminished in several experiments; in others it was somewhat reduced, due perhaps to the leakage of perfusion fluid through severed blood vessels. Of particular interest in this respect is the observation which will be described in greater detail below, that dorsal root stimulation can completely stop the effect of sympathetic stimulation. This shows clearly that all the vessels which are innervated by constrictor fibers are also supplied by dorsal root fibers.

Effect of dorsal root stimulation on the response to repetitive sympathetic stimulation. The tonic constriction of the blood vessels is, under normal conditions, largely the response to a continuous discharge of impulses transmitted through sympathetic nerve fibers (see Adrian, Bronk and

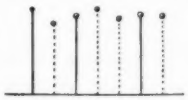

Fig. 4. Influence of dorsal root stimulation on the height of the contraction. Full lines give magnitude of contraction without, interrupted line that with simultaneous dorsal root stimulation. All the contractions produced by stimulating the sympathetic for 5 seconds.

Phillips, 1932). The dilatation produced by the nerves could therefore be explained if it could be demonstrated that the magnitude of the response to sympathetic impulses is reduced by the action of the vasodilators. This question was studied in the following way. A constriction of the blood vessels was produced by stimulating the sympathetic for 5 seconds at a frequency of 20 stimuli per second and the degree of contraction compared with that resulting from a second stimulation during which the dorsal roots were also stimulated. (Actually the dorsal root stimulation was started several seconds before that of the sympathetic on account of the longer latent period of its action.) The result of one of these experiments is given in figure 4 where the height of the contraction is represented by vertical lines. Solid lines are used for the responses to sympathetic stimulation alone and interrupted lines for the response during simultaneous dorsal root stimulation. It is seen that the latter has no measurable influence on the magnitude of the contraction.

Dorsal root stimulation also failed to have any effect on an existing maximal constriction. If the sympathetic was stimulated repetitively and

sufficient time was allowed to produce a constant and maximal contraction, dorsal root stimulation did not cause any dilatation.

Effect of dorsal root stimulation on the response to a small number of stimuli. The failure of the dorsal root impulses to diminish the response to sympathetic stimulation seemed at first surprising. The reason for this negative result was finally found in the fact that the excitatory effect of the sympathetic impulses, at the high frequency used, was so strong that it completely overpowered the inhibitory action of the dorsal roots. This is shown in the following experiments in which the sympathetic was stimulated by only one or a few shocks. In sensitive preparations one single, just maximal stimulus produces a measurable response of the blood vessel musculature. This is completely abolished by simultaneous stimulation of the dorsal roots (frequency 20 per sec.) (fig. 5). Likewise the response to two stimuli following in rapid succession (within 1 sec.) is abolished or greatly reduced. As the number of stimuli in a single burst is increased the influence of the dorsal root stimulation becomes relatively smaller.

This relationship can be expressed quantitatively if the number of stimuli

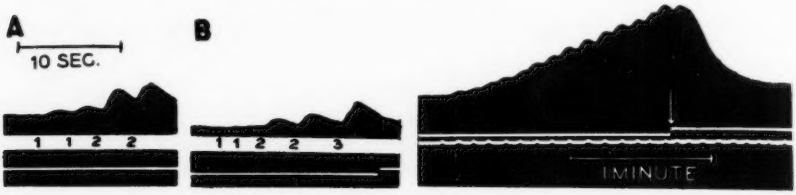

Fig. 5 Fig. 7

Fig. 5. Contractions produced by small number of sympathetic volleys; their number is indicated below each contraction. Lower signal marks dorsal root stimulation. Note that a single volley is ineffective during dorsal root stimulation.

Fig. 7. Response of blood vessels to series of sympathetic and dorsal root volleys. One stimulus applied to sympathetic about every 1.5 seconds (marked by lower signal). The arrow indicates the beginning of dorsal root stimulation (20 per sec.) (marked by upper signal).

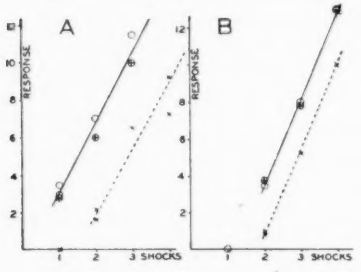

Fig. 6, A. and B. Height of contraction (expressed in millimeter deflection of the flowmeter) as a function of the number of stimuli applied to the sympathetic in a burst (within 1 sec.). Circles represent contractions without, crosses represent contractions during stimulation of dorsal roots (frequency 20 per sec.). The plain circles indicate the first series, the crossed circles the last of the 3 series of observations.

which are applied to the sympathetic in a given burst is plotted against the height of the contraction produced. In figure 6 the circles represent the contractions produced by sympathetic stimulation alone, the crosses, those produced during simultaneous stimulation of the dorsal roots. The points of the two series fall approximately on two straight lines which are parallel within the limits of accuracy. The inhibitory stimulation has the effect of shifting the line representing the relation between the number of stimuli and the magnitude of the response in the direction of a greater number of stimuli. It makes the first and sometimes the second stimulus of a burst ineffective, but on further increasing the number of shocks the response increases as rapidly as without the inhibitory impulses. If a very large number of stimuli are used one would expect the difference in the response to be very small; this explains the results cited above which failed to show any influence of the stimulation of the dorsal roots.

Although the inhibitory impulses transmitted by the dorsal roots are not able to prevent the contractions produced by a high frequency of sympathetic stimulation it is probable that the frequency of sympathetic discharge in the intact animal is usually sufficiently low for its effect to be controlled by the dorsal root impulses. In fact Bronk, Ferguson, Margaria and Solandt (1936) have recently recorded the impulses in single sympathetic fibers and found them to be of much lower frequency than those in somatic nerves. That such impulse frequencies can nevertheless produce maximal contractions by summation of the individual slow responses is shown by figure 7 where the frequency of stimulation was less than one per second. Such contractions are completely inhibited by dorsal root stimulation (beginning at the arrow) as shown in that same figure.

Discussion. From the observations just reported it may be concluded that the dilator impulses produce their effect by a definite change of the excitability of the muscles of the blood vessels. At a given frequency of dorsal root impulses a certain number of sympathetic impulses are quantitatively inactivated and the muscular activity resulting from the stimulation of the vasomotor nerves appears to be determined by the algebraic summation of the excitatory and inhibitory influences. A simple explanation of the mechanism of this interaction can be given on the basis of the humoral mediation of impulses, and it will be shown that this permits a quantitative explanation of the essential facts which are reported in this paper.

On the basis of present evidence concerning these mediators we assume that stimulation of the dorsal roots produces a dilator substance of the sympathetic fibers, a constrictor substance. We make the further assumption that a certain amount of each of these substances will just "neutralize" each other. It follows then, that the amount of constrictor

substance which is produced by a low frequency of sympathetic impulses will be made just ineffective by the dilator substance produced by the dorsal root stimulation. But if an excess amount of constrictor substance is liberated by additional sympathetic impulses a contraction will result. This describes the relation between the action of the two nerves as found experimentally.

The effect of acetylcholine on the response of the blood vessels is of interest in connection with this theory. High concentrations (1:500,000 of acetyl-β-methylcholine) suppress the response to sympathetic stimulation almost completely. Moderate concentrations (about 1:4,000,000 but very different in different preparations) suppress the response to short periods of stimulation, whereas a maximal response is still obtained by continuous excitation. In the presence of acetylcholine the response to sympathetic stimulation has a considerable latent period (fig. 8).

The theory just outlined implies that the dilator substance has no direct effect on the smooth muscle cells, but merely inactivates a certain amount

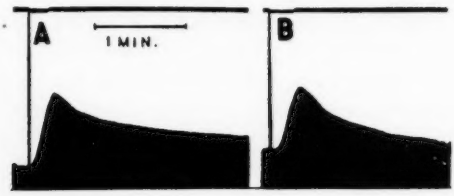

Fig. 8, A. and B. Contractions produced by stimulation of the sympathetic at a frequency of 10 stimuli per second (marked by signal). In B application of acetyl-β-methylcholine 1:4,000,000. Note the longer latent period of the response.

of constrictor substance. The fact that dorsal root stimulation can cause a dilatation in the absence of any sympathetic activity may be explained by the assumption that the tonic contraction of the blood vessel musculature is the result of a process or substance fundamentally the same as that produced by sympathetic impulses. In these respects the inhibition of smooth muscle is apparently different from the vagal action on the heart. The chronotropic effects of both cardiac nerves are exerted directly on the pacemaker. According to Rosenblueth and Simeone (1934) the percentage change of heart rate produced by each of the cardiac nerves is constant and independent of the action of the antagonistic nerve. The effects of the vasoconstrictor and vasodilator impulses on the other hand resemble more closely the c.e.s. and c.i.s. according to Sherrington's conception of the nature of reflex activity (see Sherrington, 1925, and Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932), because c.i.s. inactivates quantitatively the c.e.s. without acting on the motoneurone directly.

These observations give a simple explanation of the difference between iterative and non-iterative systems, a difference which has been stressed by Lapicque (1926) in order to explain heterochronism in the autonomic effector systems. Thus it had been assumed until recently that a succession of autonomic nerve impulses is necessary in order to elicit a response. However, non-iterative responses have been observed by Gilson (1931), Bishop and Heinbecker (1932) and by Rosenblueth (1932) and we now see that the sympathetic-constrictor system is likewise non-iterative under certain conditions. It does however become iterative under the influence of dorsal root stimulation although it might be argued that in such a case the response to single stimuli is present, but too small to be detected. This however seems improbable when we consider the relationship between the number of stimuli and the strength of the response, given above as a straight line which does not approach or go through the origin (fig. 6A).

The response is also iterative under certain experimental conditions such as at the beginning of the experiment, shortly after the start of perfusion (fig. 6B). The basis for this condition of the muscles which requires repetitive stimulation to elicit a response is probably fundamentally the same as that resulting from the action of the dorsal root impulses, namely the presence of a vasodilator substance. That this is the case is shown by the fact that the perfusate from such a preparation will produce dilatation. The perfusion fluid from a Trendelenburg preparation was collected and injected into another preparation in which the vessels were constricted; a dilatation was produced. This is in agreement with the general conceptions regarding the importance of metabolites in regulating the tone of blood vessels during and after muscular exercise. Thus Rigler (1932) has shown that the perfusion fluid collected from a Trendelenburg preparation after a strong contraction of the skeletal muscles has vasodilator effects. The action of the perfusion fluid during rest is much weaker and probably could not be detected by the usual methods.

The author is greatly indebted to Dr. D. W. Bronk and Dr. H. K. Hartline for valuable criticism.

SUMMARY

1. The vascular responses of a perfused hind leg of the frog have been observed by recording changes in the rate of flow of the perfusion fluid. A sensitive flowmeter suitable for this purpose is described.

2. The question whether vasodilator impulses diminish the response to vasoconstrictor impulses has been studied. It was found that repetitive stimulation of the dorsal roots does not measurably change the response to repetitive stimulation of the sympathetic if the frequency of the sympathetic volleys is high (20 per sec.). The constrictor effect of sympathetic

volleys at a lower frequency (0.5–1 per sec.), however, can be completely suppressed by simultaneous repetitive stimulation of the dorsal roots. As the frequency of impulses in sympathetic nerves under normal conditions is probably small, this mechanism is sufficient to explain the effect of the dilator nerves in the intact animal.

3. Different numbers of stimuli were applied to the sympathetic in rapid succession (within 1 sec.). The response increases linearly with the number of stimuli in a given burst. If the dorsal roots are stimulated simultaneously, a similar relationship is found, but the line representing the variation of the response with the number of stimuli is shifted in the direction of a larger number of stimuli. Whereas usually a single sympathetic volley produces a measurable response, the stimulation of the dilator nerves makes the system iterative.

4. The relation between the effects of the antagonistic nerves can be explained on the assumption that the action of the vasoconstrictor and vasodilator impulses is mediated by neurohormones which inactivate each other and that, consequently, the contraction produced by the stimulation of the vasomotor nerves is determined by the excess of the vasoconstrictor substance.

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EFFECTS OF ANATOMICAL SEPARATION OF THE HYPOPHYSIS FROM THE HYPOTHALAMUS IN THE DOG[1]

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Mahoney and Sheehan (1) recently reviewed in detail the literature dealing with separation or occlusion of the pituitary stalk in the dog. In all such attempts, the physiological effects were those of complete or partial hypophysectomy. Histological studies of the hypophysis substantiated the physiological result. It is clear that investigators lay the blame for the hypophyseal atrophy, which followed such attempts, to an interference in the blood supply. The ease with which the vascular supply to the anterior lobe can be disturbed by stalk separation is readily appreciated from Dandy's (2) and Basir's (3) descriptions of the vascular system of the canine hypophysis. It is generally agreed that there is a communication between the blood channels of the pars anterior and the pars nervosa by way of the sinuses of the pars tuberalis.

METHOD. Dogs in good physical condition, ranging in weight from three to ten kilograms were caged several days previous to the operation. Water was available constantly, and was measured every twenty-four hours, while food remained in the cage from 4:00 p.m. until 8:30 a.m. The food consisted of Baloration[2] which was mixed with water (60 per cent by weight) at the time of feeding. In records of water consumption, the water in the food was not included. This procedure standardized with considerable exactness the food and water intake over the respective periods. Urine was collected in covered jars and measured every twenty-four hours. Following operation the same regime was maintained until the termination of the experiment. Blood sugar determinations were made just previous to, and immediately after operations, and for several days thereafter. Rectal temperatures were recorded daily for ten days to two weeks following operation.

OPERATIVE PROCEDURE. The hypophysis was approached by the subtemporal route. After the exposure was completed, the stalk was cut

[1] Aided by a grant from the Rockefeller Foundation.

[2] Baloration is a prepared, well-balanced dog food obtained from Tioga Mills, Inc., Waverly, New York.

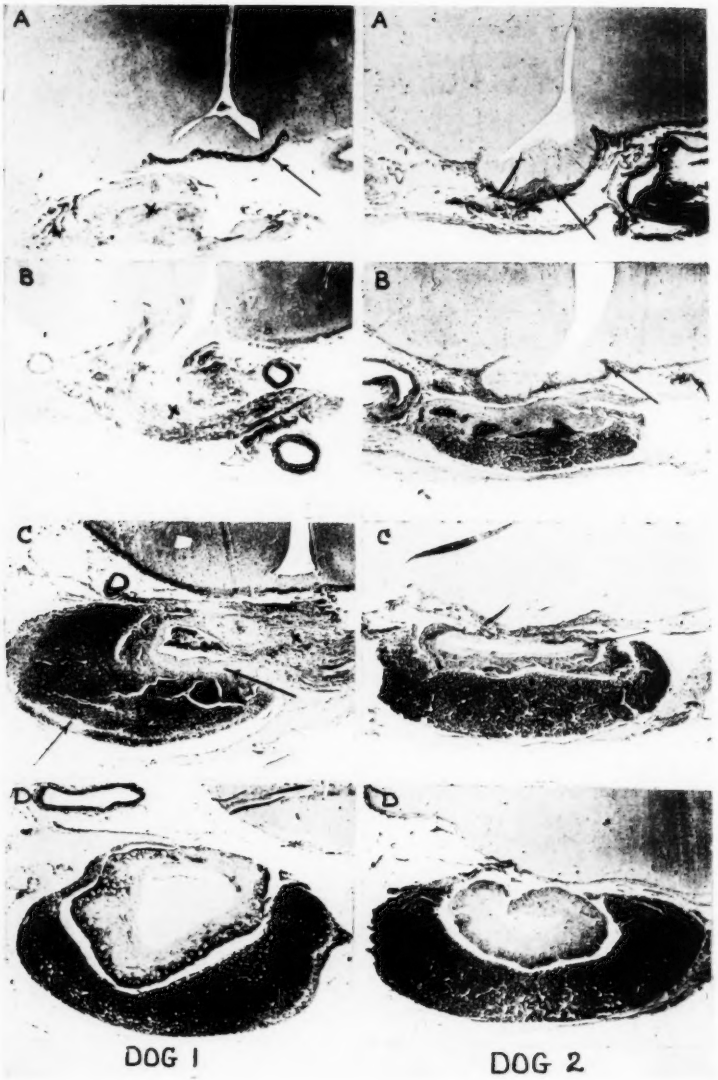

Fig. 1. Photomicrographs of serial sections. Dog 1, terminated 15th day. Note: A. The strap of pars tuberalis remaining attached to the brain anteriorly, indicated by arrow. Muscle which was inserted between separated gland and hypothalamus, indicated by *X*. B. Muscle, indicated by *X*. C. Distal portion of infundibulum, indicated by arrow; intact anterior lobe except for slight area of necrosis, indicated by arrow; pars tuberalis bounding infundibulum, and muscle

by scissors, attempting to make the section such that the whole of the gland, including the infundibulum, was severed from the hypothalamus. The successful accomplishment of this procedure has been described and illustrated elsewhere (4) as the first step in hypophysectomy.

Results. Clean severance of the hypophysis from the hypothalamus was attempted in five dogs. In two of these, a bit of muscle was placed in the line of separation, while in the other three no precaution was taken to prevent approximation of the cut surfaces. For the first few days after operation, the dogs showed a mild increase in water intake, as well as a possible trace of increased food consumption. Following this initial rise water intake fell below normal, in some cases to zero, for a few days, returning subsequently to normal or slightly above normal (see curves on dogs 2 and 3 in fig. 2). There was no significant alteration in blood sugar, rectal temperature, or in the general behavior of the animals, either directly following operation or subsequently.

Serial section of the hypophysis and hypothalamus revealed that in all animals the anterior strap of pars tuberalis remained attached to the brain base (see fig. 1, dog 1-A), and in two cases a bit of the thickened portion of the pars tuberalis remained anteriorly (see fig. 1, dog 2-A). In all instances there was a complete separation of the remainder of the gland including the greater portion of the infundibulum. In three experiments there was no macroscopic degeneration in the pars tuberalis, anterior lobe, pars intermedia, or infudibulum peripheral to the lesion. In one instance there was considerable atrophy in the anterior lobe, while in the remaining case only a trace was present (see fig. 1, dogs 1-C and D). In all cases there was a characteristic central disappearance of tissue in the infundibular process such that there was a direct continuation of the infundibular cavity (distal tip of the third ventricle) in to the infundibular process (see fig. 1, dog 1-D and dog 2-D). An increased cellularity in the tissue of the infundibular process which remained was a constant finding (see fig. 3).

In another group of four animals immediately after the gland had been severed, its proximal surface was cauterized slightly about the region of the severed stalk. This is easily possible in most instances without involvement of the hypothalamus because the gland after separation retracts somewhat into the fossa. Three of these dogs exhibited a much

plug. D. Intact anterior lobe except for small area of necrosis, and central atrophy of the infundibular process.

Dog 2, terminated 36th day.

Note: A. Tip of pars tuberalis intact on brain base anteriorly. B. A small strap of pars tuberalis intact on brain base; distal to section the pars tuberalis essentially undisturbed, and undisturbed anterior lobe. C. Infundibulum, pars tuberalis and anterior lobe all undisturbed. D. Intact pars anterior and pars intermedius, but characteristic central atrophy of infundibular process.

more marked increase in water consumption for the first few days following operation than did those of the preceding series. The temporary diabetes insipidus in these animals equalled the maximum seen in our series and ranged from 600 to 800 cc. of water per kilogram of body weight per day. After several days the water intake returned to normal or a trace above normal, where it remained until termination of the experiment. The fourth dog of this group exhibited, directly following operation, only a slight increase in water consumption which subsided to zero on the ninth day, then rose rapidly to a high level in the characteristic fashion of the permanent phase of diabetes insipidus as described by the Ranson group

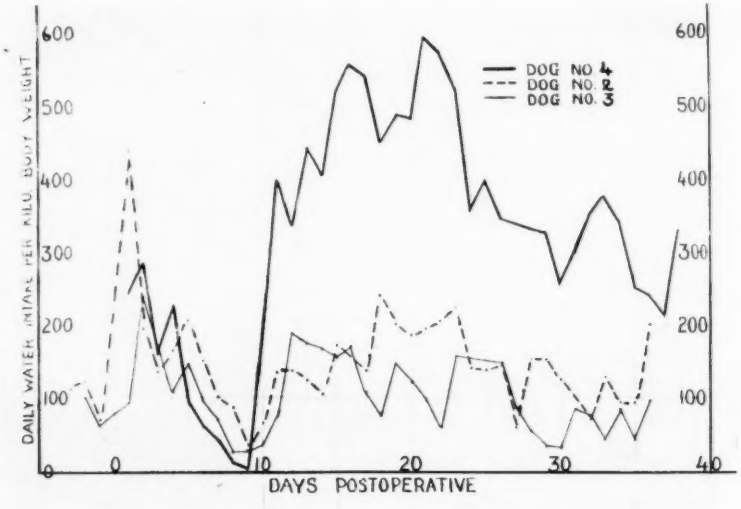

Fig. 2. Dogs 2 and 3 had the stalk cut, without detectable injury to gland as illustrated in figure 1. In dog 4 subsequent to cutting the stalk the gland was cauterized slightly about the area of entrance of the stalk into the gland.

(5) (see fig. 2, curve dog 4). The food intake of this group of animals, though it increased slightly following operation in two animals, remained essentially normal. In these two dogs a definite tendency to adiposity was evident.

Macroscopic study of serial sections of the hypophysis revealed varying degrees of involvement of the pars tuberalis and pars anterior. In all instances, however, considerable tissue of each category remained. In the case of dog 4 there was very little infringement upon the pars tuberalis and none upon the pars anterior. In all experiments there was a hollowing out of the infundibular process with an increased cellularity in the

remaining portion. In no case did the pars intermediate seem encroached upon. The whole of the gland, including the infundibulum, was separated in dogs 5 and 4. Dog 5 exhibited no deviation from the normal subsequent to the tenth day.

Discussion. It is readily apparent from the photographs shown in figure 1 that an adequate blood supply to the anterior lobe of the canine hypophysis remains after a clean separation of the gland from the hypothalamus. Further, the cautery experiments demonstrate that considerable encroachment upon the pars tuberalis is possible without evident disturbance in the vascular supply to the remaining portion of the gland. It is to be stressed that such a surgical result was to be expected from the description of the vascular supply of the gland as given by Dandy and Basir. It is evident that in previous attempts at severance or occlusion

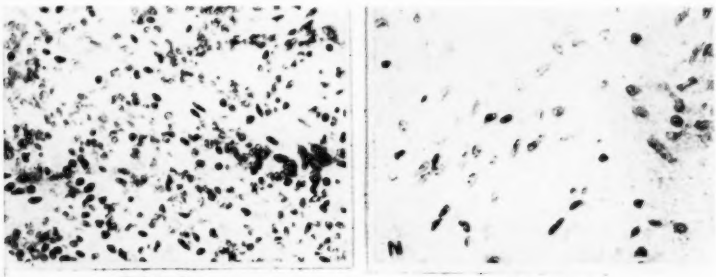

Fig. 3. Right is a photomicrograph taken from the infundibular process of an unoperated dog.

Left is a photomicrograph taken from the infundibular process remaining in dog 2. Both sections were stained with cresyl violet and have same magnification.

of the stalk in the dog the anastomosis through the pars tuberalis has been greatly encroached upon.

The changes which occurred in the infundibular process appear to be equivalent to those described by Fischer, Ingram, Hare and Ranson (4) in the cat following hypothalamic lesions. These investigators attribute the changes to section of the nerve fibers passing to this structure from the anterior hypothalamus. Although we have encountered these results following hypothalamic lesions, such that these fibers would have been cut, we are not certain but that a mechanical factor is in part responsible.

The absence of increased water consumption following clean stalk sections in our experiments demonstrates that denervation of the infundibular process is not the cause of the diabetes insipidus which follows hypothalamic lesions as has been postulated by the Richter and Ranson schools. Furthermore the precipitation of the permanent phase of dia-

betes insipidus by trauma to the proximal portion of the separated gland demonstrates that the essential mechanism necessary for the production of both the temporary and permanent phases of diabetes insipidus is inherent in the hypophysis itself.

We hasten to point out that in the dog, as well as in the cat, diabetes insipidus of varying severity frequently follows bilateral lesions placed with the needle in the hypothalamus. The incidence in the cat is strikingly less than in the dog. The curve on dog 5 in figure 4 illustrates such

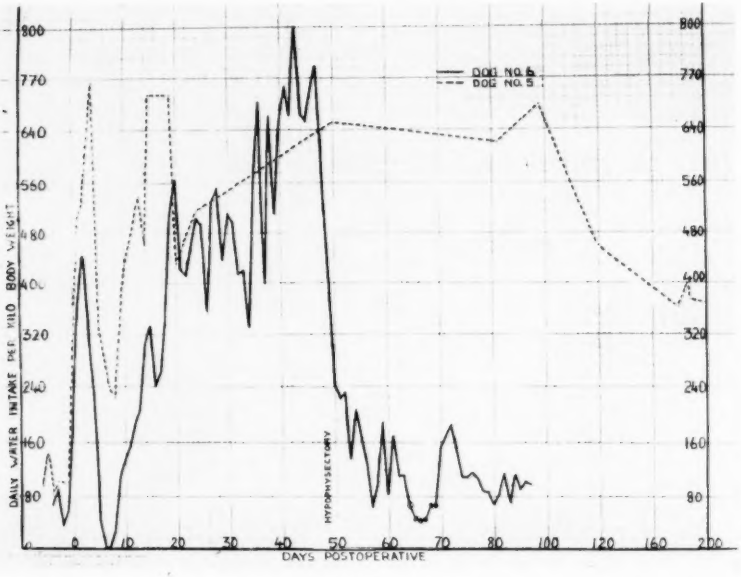

Fig. 4. Both experiments are described in the text. In dog 6, following hypophysectomy, some milk was given in addition to water and the total fluid intake is graphed. The values circled represent water intake on days when milk was not given and are therefore directly comparable with values graphed previous to hypophysectomy.

an experiment. If diabetes insipidus following such hypothalamic lesions is not the result of hypophyseal denervation, what then is the mechanism concerned? In the light of the foregoing results a neighboring involvement of the hypophysis would seem more probable than the possibility of an additional independent neural mechanism located in the hypothalamus.

That the hypophysis is essential for the diabetes insipidus induced by a hypothalamic lesion is evidenced by the curve on dog 6, shown in figure 4. In this dog hypophysectomy, on the 49th day after the central lesion

was placed, eliminated abruptly a striking diabetes insipidus. This experiment does not necessarily demonstrate that the direct cause of the increased water consumption produced by the hypothalamic lesion was due to a disturbance in the gland, because the effect of the hypophysectomy on metabolism might be sufficient to eliminate the diabetes insipidus.

Exactly the same paradox exists relative to enhanced appetite and resultant adiposity. Enhanced food consumption occurs in instances following *1*, hypothalamic lesions (6), and *2*, removal of the posterior lobe of the hypophysis (7), yet stalk section has been accomplished with no disturbance in food consumption or body weight. Immediately following operation there is a marked increase in the amount of food eaten and for the first three or four weeks there is a progressive rapid rise in body weight. In severe cases food intake is limited by the capacity of the stomach, food being repeatedly regurgitated, then eaten again. Eventually food intake returns to normal and body weight is in turn either maintained or returns slowly to normal. To this degree there occur here also the temporary and permanent phases. It is to be recalled that a tendency to adiposity occurred in two instances where the hypophysis was encroached upon by cautery, also a typical case of enhanced appetite with adiposity has been encountered (8) following gland separation where the hypophysis was encroached upon by pressure with forceps.

SUMMARY

The hypophysis was successfully separated from the hypothalamus *1*, without infringement upon the adequacy of the blood supply to the gland, or *2*, without inducing enhanced food or water consumption.

Experiments are described and recalled which demonstrate that enhanced food intake and diabetes insipidus can be produced by involvement of the hypophysis alone.

An experiment is described and illustrated wherein a severe diabetes insipidus elicited by a hypothalamic lesion was eliminated abruptly by hypophysectomy.

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THE MEASUREMENT OF SERUM VOLUME

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In estimating the quantity of any component present in blood serum, the measurement of serum volume is obviously essential. The methods for this measurement, however, have not given entirely satisfactory and reproducible results. The results with different methods and with the same method by different investigators have conspicuously shown lack of agreement. Of the various methods devised, the dye method has been the one most generally employed. In this method a solution of a nontoxic dye, such as brilliant vital red, is injected intravenously into a subject and from its dilution in the serum, the volume of the serum is estimated.

The method originally proposed by Keith, Rowntree, and Geraghty (1) and later modified by Smith, Whipple and associates (2) has been discussed by Erlanger (3), Lamson and Rosenthal (4), Peters and Van Slyke (5) and others. It has been pointed out that in the measurement of serum volume by the dye method the main discrepancies are concerned first, with respect to the accuracy of measuring the dye which is introduced; second, the accuracy with which the blood sample obtained after injection represents a uniform mixing; third, the extent to which the dye remains in the circulation during the time taken for mixing; and fourth, the accuracy of the colorimetric measurement. The studies which are here reported are directed chiefly toward the consideration of these problems.

Colorimetry of vital red. Gregerson, Gibson and Stead (6) have indicated that one of the chief problems in the measurement of serum volume by means of the dye method concerns itself with the colorimetry of vital red. Inasmuch as vital red dissolved in normal saline and added to serum yields a different color value than when it is dissolved in water and added to serum, studies were made to determine the nature of the color produced by vital red in solutions of different ionic concentrations, of different ion species and in the presence of serum.

Apparent differences of concentration of 0.004 per cent vital red in water and salt solution at various pH values with Wratten no. 74 and no. 75 filters.

In table 1 is shown the composition of various solutions of dye in water, phosphate buffer, NaCl, KCl and NaI solution. The solutions all containing the same concentration of dye were compared in a colorimeter using in the lens system either a no. 74 Wratten filter with transmission as shown in figure 1, maximal at λ530 mμ and falling to 10 per cent of maximal at λ510 and 560 mμ, or a no. 75 Wratten filter with transmission maximal at λ485 mμ and falling to 10 per cent of maximal at λ465 and 515 mμ. A is the solution of dye in water and its apparent concentration is taken as unity for comparison with the other solutions. B is the dye in phosphate buffer, pH = 8.0; D and E, in NaCl solution; C, F and H, in NaCl plus buffer; G, in NaI plus buffer; I, J and K in KCl plus buffer;

TABLE 1

Influence of salts on the colorimetric reading of 0.004 per cent vital red in water

DESIGNATION	pH	IONIC STRENGTH	$\frac{Na}{M}$	$\frac{K}{M}$	$\frac{Na+K}{M}$	$\frac{Cl}{M}$	$\frac{I}{M}$	$\frac{H_2PO_4^-}{M}$	$\frac{HPO_4^=}{M}$	APPARENT FILTER #74	CONCENTRATION FILTER #75
		μ									
A	?									1.00	1.00
B	8.0	0.029	0.019	0.0005	0.020			0.0005	0.010	0.94	
C†	8.0	0.150	0.109	0.002	0.111	0.030		0.002	0.039	0.83	
D	?	0.150	0.150		0.150	0.150				0.86	
E	?	0.300	0.300		0.300	0.300					0.77
F*	8.0	0.400	0.359	0.002	0.361	0.280		0.002	0.039	0.79	
G	8.0	0.400	0.359	0.002	0.361		0.280	0.002	0.039	0.69	
H	5.9	0.150	0.050	0.090	0.140	0.030		0.090	0.010	0.74	
I	5.9	0.379	0.079	0.290	0.369	0.259		0.090	0.010	0.60	
J	8.0	0.389	0.079	0.271	0.350	0.269		0.002	0.039	0.61	
K†	8.0	0.400	0.079	0.282	0.361	0.280		0 002	0.039	0.58	
L	?	0.300		0.300	0.300	0.300					0.62

* Average reading of two solutions of identical composition.

† Average reading of three solutions of identical composition.

and L, in KCl. Comparison of A, B, C and F with no. 74 filter shows decreasing color intensity as Na^+ increases. Comparison of C and D indicates that Cl decreases the color less than $HPO_4^=$. Comparison of F and G indicates that Cl decreases the color less than I^-. Comparison of E and L and of F and K indicates that, with either filter Na^+ decreases the color less than K^+. Comparison of H and I, with no. 74 filter, indicates decreasing color with increasing K^+. Comparison of I and J indicates that if Na^+ and K^+ are kept approximately constant, change in pH has little effect on color intensity.

Apparent concentration of dye added to serum with or without added salt with Wratten no. 74 and no. 75 filters. In table 2 is shown a series of additions of dye to 0.004 per cent concentration in 75 per cent dog serum

in water (M) or in NaCl or KCl solution (N to R). With both filters the color of the dye added to serum, without added salt (M) is taken as unity. For expressing ionic strength and Na^+ and K^+ concentrations the serum was assumed to contain 140 mM/L Na^+, 5 mM/L K^+ and $\mu = 150$. N, O and P were prepared with added KCl; Q and R, with added NaCl. With a no. 74 filter both KCl and NaCl increased the apparent concentration of the dye instead of diminishing it, as in the absence of serum. In the presence of serum Na^+ was more effective than K^+ in producing this change. With a no. 75 filter the changes are very slight, within the error of measurement.

TABLE 2

Influence of salts on colorimetric reading of vital red in serum

DESIGNATION	IONIC STRENGTH	$\frac{Na}{M}$	$\frac{K}{M}$	$\frac{Na + K}{M}$	APPARENT CONCENTRATION	
0.004 per cent dye in 75 per cent dog serum					Filter #74	Filter #75
	μ					
M	0.113	0.105	0.004	0.109	1.00	1.00
N	0.123	0.105	0.014	0.119	1.00	1.00
O	0.213	0.105	0.104	0.209	1.05	1.00
P	0.313	0.105	0.204	0.309	1.14	1.01
Q	0.213	0.205	0.004	0.209	1.14	1.01
R	0.313	0.305	0.004	0.309	1.19	1.01
0.0066 per cent dye in 75 per cent human serum						
S	0.113	0.105	0.004	0.109		1.00
T	0.277	0.269	0.004	0.273		0.97

With 75 per cent human serum containing 0.0066 per cent dye the addition of NaCl slightly reduced the apparent concentration with a no. 75 filter.

The change of color of vital red with time in aqueous solution (0.002 to 0.005 per cent) was tested with both a no. 74 and no. 75 Wratten filter, reading against a neutral tint filter with an optical density of 1.0. The maximum variation with the no. 74 filter was 0.2 mm. and with the no. 75, 0.1 mm. in a total reading of about 10 mm. over a period of $1\frac{1}{2}$ hours. A consistent weakening of color (0.1 to 0.3 mm.) was observed after 18 hours.

Spectrophotometry of vital red solution in water and in 75 per cent dog's serum with or without added NaCl or KCl. All solutions represented in figure 1 were prepared to contain 0.0040 per cent or 0.0024 per cent dye in the following solvents: W, water; Na W, 0.3 M NaCl solution; K W,

0.3 M KCl solution; S, 75 per cent dog serum diluted with water; NaS, 75 per cent dog serum with aqueous solution of NaCl added to 0.2 M in addition to the salt of the serum. C S represents the reading of 75 per cent dog serum without added dye. The solutions were read in a 1 cm. cell with a Bausch and Lomb spectrophotometer of the König-Martens type with the technique of Drabkin and Austin (7). In reading W, Na W and K W the compensating cell contained water. In reading S and Na S the compensating cell contained 75 per cent serum without dye; and the total absorption would accordingly be the sum of each of these curves with the curve *C S* in figure 1. The extinction coefficient is calculated

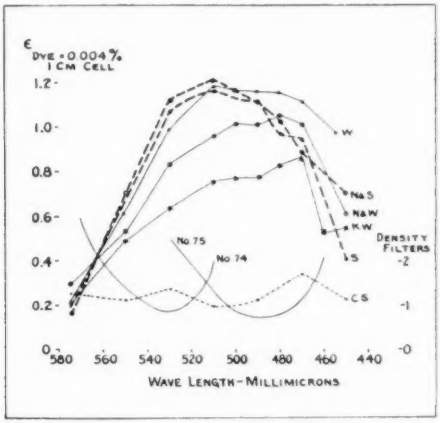

Fig. 1. The influence of salts and serum on spectral absorption of vital red (0.004 per cent solution). *W*, dye in water; *NaW*, dye in 0.3 M NaCl; *KW*, dye in 0.3 M KCl; *S*, dye in 75 per cent dog's serum diluted with water; *NaS*, dye in 75 per cent dog's serum in aqueous NaCl to give final concentration of 0.2 M NaCl in addition to the salt of the serum; *CS*, 75 per cent dog's serum diluted with water, no dye. Curves 74 and 75 show the optical density of the Wratten filters employed.

for 0.004 per cent vital red in 1 cm. cell. The same graph shows the optical density of the no. 74 and no. 75 Wratten filters used with the colorimeter. The curves show the depressed absorption of the dye present in the NaCl solution to that of water, and the still greater effect on adding a normal KCl solution. They show displacement of the curve toward the red on adding serum and slight increase of absorption on adding NaCl to the serum. These observations are consistent with the colorimetric observations reported, and the greater consistency of readings with the no. 75 filter as compared with the no. 74 could be predicted.

These studies indicate the importance of the type of standard originally recommended by Keith, Rowntree and Geraghty, namely, one in which

the vital red is dissolved in the serum of the patient to be tested. The difficulty arising from a ratio of dye to serum in the standard differing from that in the unknown, hence preventing perfect matching, can be readily obviated if the apparent color is a linear function of concentration of dye added to serum over a range that includes all of the unknowns. If two standards be prepared by adding dye to the serum obtained before

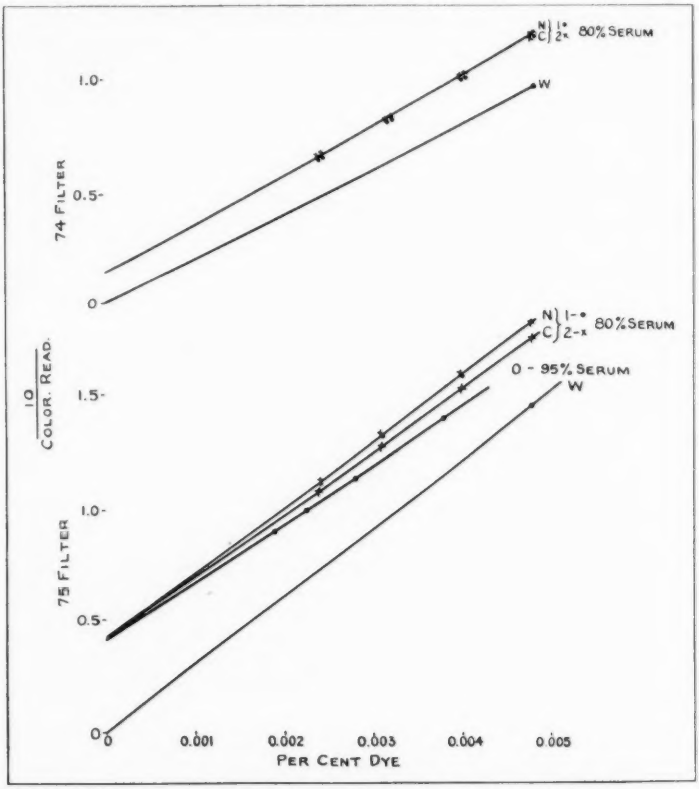

Fig. 2. Linear increment in light absorption as vital red is added to serum. *N*, *C* and *O*, dye added to human serum (see text). *W*, dye added to water.

injection of dye so that one standard is weaker than the weakest unknown and the other standard stronger than the strongest unknown, the reciprocals of the colorimeter readings obtained with a neutral tint standard and an appropriate color filter might furnish a line on which might be interpolated the reciprocals of the readings of any of the unknowns to determine the concentration of dye present.

Figure 2 shows a test of this on five human sera, two bleedings an hour apart on subject *N*, two bleedings similarly on subject *C* and one bleeding on subject *O*. With the first four sera to 2 cc. portions was added 0.5 cc. of varying concentrations of vital red in water to give final concentrations shown on the abscissae. Each of the 16 resulting mixtures was read with both the no. 74 and no. 75 Wratten filter. With subject O, to 2 cc. portions of serum was added 0.1 cc. of varying concentration of dye in water. In addition a reading with each filter was taken of 0.0048 per cent dye in water, *W*. The final concentration of serum for N and C was 80 per cent; for O, 95 per cent. The absorption of the diluted sera without dye was read with the no. 75 filter but was too weak to read with the no. 74 filter. It is evident that the relations are satisfactorily linear. With the no. 75 filter the slope in serum is a trifle less steep than in water.

TABLE 3

Influence of hemoglobin on colorimetric reading of vital red (No. 75 Wratten filter)

SERUM MIXTURE*	CONCENTRATION OF HEMOGLOBIN	COLORIMETRIC READINGS #75 FILTER	POSITIVE BENZIDINE REACTION
	mgm. per 100 cc.	*mm.*	*dilution*
1	0.0	5.4	1 to 10 with NSS
2	10.5	5.4	1 to 50 with NSS
3	21.0	5.1	
4	42.0	5.1	
5	84.0	4.7	

* The serum mixtures were prepared by adding to 4 cc. of serum, 0.5 cc. vital red in aqueous solution and 0.5 cc. of hemoglobin solutions of various concentrations prepared from hemolyzed cells. All mixtures contained 0.0048 per cent final concentration of vital red.

Thus far little superiority is indicated for one of the two color filters over the other. However, a matter of great importance for this colorimetry is interference arising from traces of oxyhemoglobin contaminating the serum. As one of the two bands of this pigment is at λ540 mμ with rapid falling off of the absorption toward λ490 mμ it can be predicted that the no. 75 filter will give results less sensitive to disturbance from traces of hemoglobin. A trace of hemoglobin so small that bands are barely visible with a small spectroscope increased the apparent concentration of the vital red using the no. 74 filter by 33 per cent, whereas with the no. 75 filter the increase was only 7 per cent. It is evident, however, that even using the latter filter, precautions must be taken to exclude disturbing contamination of the sera with traces of hemoglobin.

Vital red measurement in serum containing hemoglobin. Experiments were undertaken to determine the critical concentration of hemoglobin

that might be present in serum containing vital red and yet still be blocked out with the use of the no. 75 Wratten filter. For the colorimetric readings a neutral gray glass with an optical density of 1.0 was employed as the standard. The results of these analyses are given in table 3. It will be seen that hemoglobin present in serum containing vital red up to at least a concentration of 10.5 mgm. per 100 ml. had no influence upon the colorimetric readings. Employing the benzidine test according to the procedure of Lyle, Curtman and Marshall (8), a positive reaction was obtained when serum was diluted 1 to 10 with normal salt solution and

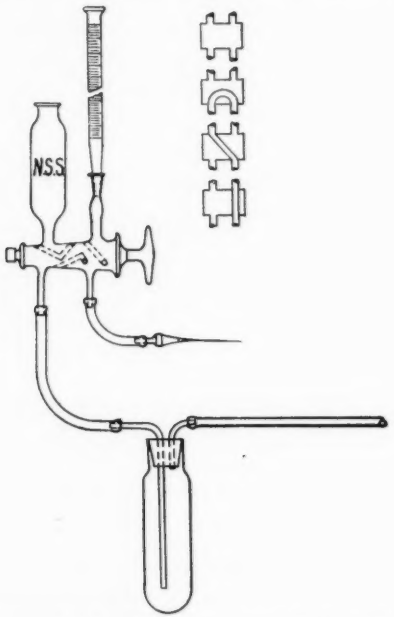

Fig. 3. Apparatus for the intravenous injection of vital red

a barely detectable reaction was obtained in a 1 to 25 dilution. In the serum to which hemoglobin was added to give an added concentration of 10.5 mgm. of hemoglobin per 100 ml., a positive benzidine reaction was obtained in a 1 to 50 dilution and a barely detectable reaction was obtained in a 1 to 200 dilution with normal salt solution. Thus it was possible to measure vital red in serum containing added hemoglobin when that serum gave a positive benzidine reaction in a dilution 5 times greater than was necessary to give the same reaction in serum not containing added hemoglobin.

With the method of double centrifugation of serum described below

positive benzidine reactions are obtained only in dilutions not exceeding 10- to 25-fold. Any specimen of serum should certainly be excluded for the purpose of this technique if it reacts positively to benzidine in 5 times this dilution. In our experience the various specimens of sera from the same individual have been closely comparable in their benzidine reaction.

Method for measurement of serum volume. The modifications we have devised for the estimation of serum volume permit of precise measurement of the dye introduced into the vein; allows adequate time for homogeneous mixing; takes into account the rate of disappearance of the dye from the serum; and permits of an accurate colorimetry of the dye present in the serum regardless of species.

The measurement is made under basal conditions after the subject has rested in bed for 30 minutes before the introduction of the vital red. The dye is introduced by means of the apparatus shown in figure 3. This apparatus consists of a four-way glass stopcock, a reservoir for normal salt solution, a burette for the dye, and a removable centrifuge tube for the collection of the initial sample of blood. The needle is placed into the vein and the blood is collected into the centrifuge tube by gentle suction. The blood remaining in the rubber tubing between the needle and the stopcock is washed back into the vein with the normal salt solution. The dye, accurately measured from a small burette graduated in 1/50 cc., is then introduced. It has been found convenient to introduce 1 cc. of a 2 per cent solution of vital red per 10 kgm. of body weight. The dye remaining in the tubing is finally washed completely into the circulation with normal salt solution.

At intervals of 30, 60 and 90 minutes after the introduction of the dye, 5 or 6 cc. samples of blood are removed without stasis from the opposite arm. The blood is allowed to stand at room temperature for at least 15 minutes, then the clot is *gently* separated from the sides of the centrifuge tube by means of a glass rod. The serum is removed after the clotted blood has been centrifuged, and the serum is again *re-centrifuged* in order to separate any erythrocytes which occasionally are carried over from the first separation. The concentration of dye present in each sample of serum is then determined.

To 2 cc. amounts of the serum obtained before the introduction of dye are added 0.5 cc. amounts respectively of 0.01 and 0.02 per cent of an aqueous solution of vital red. These solutions are then read directly in a micro colorimeter employing a neutral gray standard with an optical density of approximately 1.0 in place of the colorimeter cup and a double thickness no. 75 Wratten green filter in the lens system. An incandescent 100 Watt Tungsol headlight bulb in a Bausch and Lomb attachable illuminator is employed as the source of light. A plot of the reciprocal of the colorimeter readings against the concentrations of dye present is

made, and a straight line through the points is used to estimate the concentration of dye from the colorimetric readings of given samples taken after injection. To 2 cc. amounts of the sera obtained after the introduction of dye, 0.5 cc. of water is added. The readings of the dye present in the samples are made using the same neutral gray standard. The concentration of dye present in each sample is then obtained by interpolating the reciprocals of the colorimeter reading on the plot of the known concentrations of dye added to the original sample of the same patient's serum.

The concentration of dye in the samples of serum obtained at intervals of 30, 60 and 90 minutes gives in our experience a linear relationship. If we assume that this justifies linear extrapolation back to the moment of injection we can estimate the concentration of dye that would have been

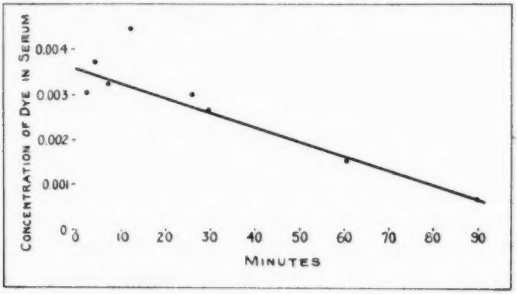

Fig. 4. Concentrations of vital red in peripheral blood serum from 2 to 90 minutes after injection. For explanation see text.

obtained at this moment were complete mixing instantaneous. Having obtained this value the serum volume may then be calculated.[1]

Selection of time intervals. In preliminary studies it was noted that the simultaneous removal of blood from both arms within the first twenty minutes after the introduction of the dye did not give the same concentrations of dye in the two samples, whereas after about twenty minutes, the same concentrations were obtained. In figure 4 is given an example of the concentrations of dye obtained at varying time intervals after the injection of dye into a dog. The scattering obtained during the first twenty minutes is demonstrated.

It might be assumed that the rate of disappearance of dye would be directly related to its concentration and that the disappearance would

[1] In order to correct for inadequate mixing and the disappearance of dye from the circulation, Erlanger in 1921 suggested that it might be possible to determine the concentration of dye at the time of injection by means of a backward extrapolation from the latter parts of the curve of disappearance.

be more rapid at first, following perhaps a semilogarithmic curve. The concentrations of dye at 30, 60 and 90 minute intervals when plotted semilogarithmically have given practically the same extrapolated values as when plotted arithmetically, the change in concentration over the period studied being too small to distinguish which type of curve is followed.

The serum volume in normal and pathological conditions. In figure 5 are given nine measurements of the serum volume in 5 normal male students between the ages of 20 and 25 years. The four duplicate determi-

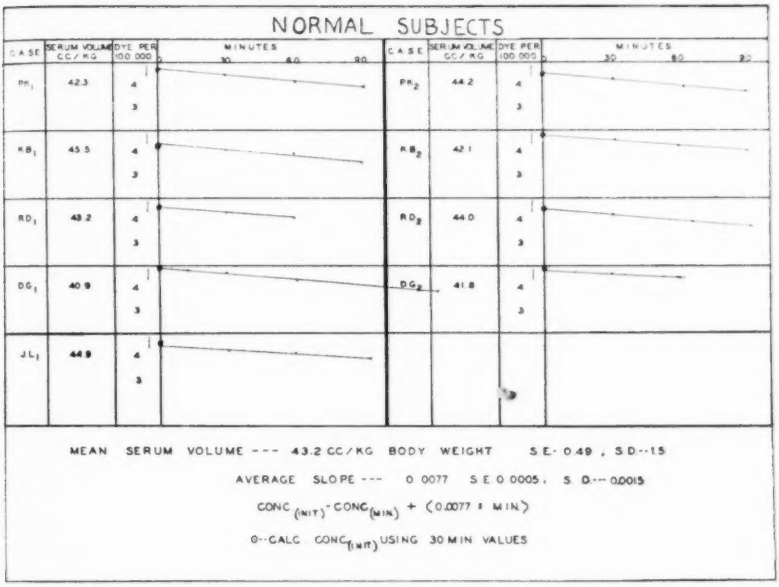

Fig. 5. The measurement of serum volume in normal individuals. For explanation see text.

nations were made at intervals of a week or more. The observed concentrations are shown at 30, 60 and 90 minutes and the estimated initial concentration in parts per 100,000 by the line extrapolated to 0 minute. The vertical lines represent the observed range of these nine normals obtained when 1 cc. of the 2 per cent dye per 10 kgm. of body weight was injected. In the second column are given the values for the serum volume, calculated as cubic centimeters of serum per kilogram of body weight. The mean of these nine determinations is 43.2 cc. with S.D. of 1.5 cc. indicating as the normal range about 40 to 46 cc. per kilogram of body weight.

The average slope of the concentration curves is 0.0077 cc./kilo body weight/minute with S.D. of 0.0015. The constancy of this slope in normal individuals is sufficient to permit calculation of the initial concentration of dye in the serum from the concentration in a single sample obtained at 30 minutes after injection by the use of the following equation:

$$\text{conc.}_{(\text{initial})} = \text{conc.}_{(\text{min.})} + (0.0077)(\text{min.})$$

The initial concentrations calculated from this equation and from the 30 minute values are represented by the open circles at 0 minutes.

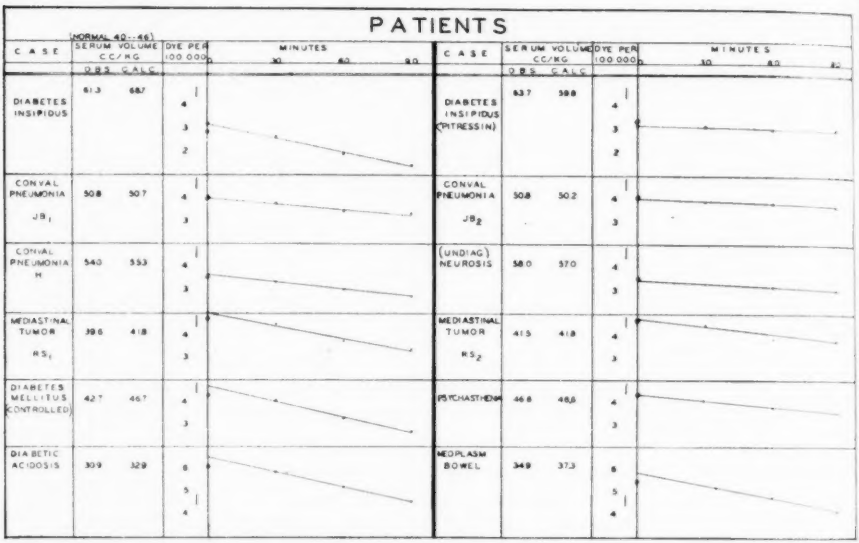

Fig. 6. The measurement of serum volume in some miscellaneous pathological conditions. For symbols see text relating to figure 5.

In figure 6 are given the measurements of the serum volume in a few patients suffering with various pathological conditions. This graph serves to demonstrate the scattering in the values which may occur pathologically. High values were obtained in one patient with diabetes insipidus and in three convalescing from pneumonia; low values have been obtained in one patient with diabetic acidosis and in one with malignancy of the bowel. The values indicated on the graph by the extrapolated line have been obtained by the three point method; the calculated values indicated by the open circle at 0 minute have been obtained by the equation using only the 30 minute values. It will be seen that the ob-

served and calculated values show sufficient agreement to render the one point method of value when repeated puncture is undesirable.

Since our measurements have all been made on serum, the question arises whether the concentration of dye in the serum is changed in the process of clotting. Hooper and his associates (9) have already demonstrated that vital red remains completely in the plasma and is not taken up by the erythrocytes. The determination of the concentration of dye in serum from a sample of blood which had been allowed to clot and the calculation of the concentration of dye in the same sample of blood to which isotonic $Na_2C_2O_4$ had been added, showed no significant difference.

It should be pointed out that there is no method available for determining whether the results with this procedure represent the absolute serum volume; indeed, the definition of absolute serum volume would be open to debate. The method, however, gives consistent reproducible results in normal individuals and reveals difference beyond the normal range in some pathological cases and thus should be of practical value.

Our sincere thanks are expressed to Dr. Norman P. Shumway who assisted one of us (FWS) in some of the preliminary studies.

SUMMARY

The studies demonstrate a marked change in the color values of vital red dissolved in aqueous solutions of salts of different ionic strength and ion species, with altered effects in the presence of serum.

A technique of colorimetry has been developed in which two standards are prepared by adding dye to serum obtained from the subject before injection of dye, one standard weaker than the weakest unknown, and the other standard stronger than the strongest unknown. The standards are compared in a colorimeter against a neutral tint filter having an optical density of 1.0 and with a double thickness no. 75 Wratten filter in the lens system. A straight line drawn through the reciprocals of these readings furnishes a valid basis for interpolation of reciprocals of the unknowns similarly read, and from this may be determined the concentration of dye in these unknowns.

A method of using the benzidine reaction has been developed for the purpose of detecting the presence of hemoglobin in serum containing vital red.

In the determination of the serum volume by the intravenous injection of vital red, there is evidence to suggest that complete mixing of the dye in the circulating serum does not occur for twenty to thirty minutes after the injection. If samples of serum are obtained under basal conditions at intervals of 30, 60 and 90 minutes after the introduction of the dye, a linear relationship is obtained which permits extrapolation to the

moment of injection and this furnishes we believe the best available estimate of the concentration of dye that would have been obtained were complete mixing instantaneous.

The quantity of dye injected is measured with precision by means of an especially constructed apparatus.

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THE EFFECT OF LACTATION AND EXERCISE ON THE RATE OF INVOLUTION OF THE UTERUS IN THE RAT

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Although obstetricians generally believe that nursing promotes the involution of the uterus in the human mother (1–12), we have been unable to find satisfactory data proving this belief. In the rat Kurmitsu and Loeb (13) measured the thickness of sections of the uterine wall at various intervals after parturition in non-lactating, lactating, castrated and lactating, and castrated and non-lactating animals. They concluded that lactation and castration hasten involution, and that the effect of lactation is exerted directly on the uterus and not indirectly through the ovaries. A report of the effect of exercise on involution of the uterus has not been found. This work was undertaken to determine the effect of lactation and exercise on the rate of involution of the uterus by actually weighing the uterus sixteen days after delivery in lactating rats, non-lactating-resting rats, and non-lactating-exercised rats.

METHODS. Albino rats from the same colony and over 100 days old were used. None of the rats had been pregnant prior to these experiments. They were fed the Steenbock diet ad libitum with the addition every two or three days of fresh carrots and beef liver. Litter mates were divided between either two of three groups, namely, a lactating group, a non-lactating group, and a non-lactating-exercise group in about one-half of the instances, or in so far as was practically possible. The rats in the lactating group were permitted to nurse all their pups. The pups were removed from the non-lactating group as soon after parturition as possible. The pups were removed from the non-lactating-exercise group and their mothers subjected to 20 minutes of swimming (water, 20°C.) in two ten minute periods daily, care being taken to dry them carefully after the swimming exercise. The exercise periods were not longer than 10 minutes because we did not care to exhaust the animals; even ten minutes tired them considerably. Floating was prohibited, and we believe the exercise was more standard than could be obtained by a tread-mill method.

The rats were killed 16 days after parturition and during the period of dioestrus. In the absence of lactation, oestrus appeared early (13, 14)

and in some instances on the sixteenth day, which necessitated the discarding of some animals. The stage of oestrus was determined by the vaginal smear method and further checked by examining the lumen of the uterus. Only animals in dioestrus have been admitted to our data. Rats of various weights were employed to ascertain any difference that might arise from the weight of the animal at the onset of its first pregnancy.

The uterus was removed by sectioning at the tubes and at the vagina near the cervix. It was then fixed for one day in a ten per cent neutral solution of formalin in 0.9 per cent sodium chloride. Then the extraneous tissue was dissected away; the uterus was carefully dried with filter paper and weighed to within 5 mgm. The body weight of the rat was determined at intervals prior to and after mating, and after death by ether.

We have arranged the data shown in the tables in order of the body weights at autopsy, rather than in the order of the weight at mating because the correlation between the uterine : body-weight ratios in the various body-weight groups was closer when the data were arranged according to the final body weight. This might appear to be obvious, but it had to be examined because of the variable effect of pregnancy and lactation, or both, on the body weight of the rats (15).

Our exercise periods were determined in the following manner: The time of involution of the uterus of a nursing rat is seven days (that for a non-nursing rat from 21 to 28 days), after which significant changes do not occur. The average time for the uterus of a nursing human mother is usually given as six weeks. The ratio between the two (7:42) is one-sixth. Hence, we thought that ten minutes of exercise in the rat should be equivalent, in regard to rate of uterine involution, to sixty minutes in the human. Since we should not consider it practical to exercise a human mother (after the period of hospitilization) fairly vigorously more than one-hour daily in one-half hour periods separated by a rest period, we exercised a group of rats (10) for ten minutes—two five-minute exercise periods—which had no effect on the rate of uterine involution. We then increased the exercise to two ten-minute periods, obtaining the results reported later. Twenty minutes of exercise in the rat would be equivalent to two hours in the human.

Results. *The effect of lactation.* The data showing the effect of lactation on the rate of involution of the uterus are given in table 1. It matters not how the data are grouped or analyzed, lactation is found to markedly augment the rate of involution. At 16 days post-partum the average weight of the uterus of the lactating rat is approximately 58 per cent less than the average weight of the uterus of the non-lactating-resting control rats.

It is to be noted that the non-lactating rats during pregnancy and the

TABLE 1

NON-LACTATING (25), RESTING RATS					LACTATING (39) RATS					NON-LACTATING, EXERCISED (31) RATS					
Rat number	Litter number	Body weight before pregnancy	Body weight at 16 days	Weight of uterus	Rat number	Litter number	Body weight before pregnancy	Body weight at 16 days	Weight of uterus	Rat number	Litter number	Body weight before pregnancy	Body weight after delivery	Body weight at 16 days	Weight of uterus
		gm.	gm.	mgm.			gm.	gm.	mgm.			gm.	gm.	gm.	mgm.
57	11	190	224	250	5	10	220	220	85	23a	3	180	200	210	225
59	8	200	222	270	7	11	220	220	100	29b	13	190	—	204	210
66	7	200	214	270	4a	—	172	212	110	20c	10	190	160	200	220
61	5	198	214	345	4	10	210	210	90	28b	11	176	—	198	210
52	—	190	214	285	5a	3	150	210	105	24c	11	176	164	194	275
50	12	200	218	330	8	6	218	210	100	4b	7	160	200	194	255
6	8	200	212	220	6a	8	200	210	100	28c	7	162	190	190	235
31	8	190	210	200	2a	8	170	208	105	12c	9	150	172	188	250
43	3	180	210	280	3a	—	177	204	105	25a	7	154	180	186	150
11	8	200	209	160	3	10	200	200	95	3b	11	180	198	186	230
60	6	200	200	220	22	8	192	200	95	15b	12	172	184	186	225
					29	8	190	200	160	24a	8	152	124	184	175
Group A ↑															
					53	6	192	200	115	1b	13	146	184	184	210
Ave. A.	7.6	195	213	257	Ave. A.	8.0	193	208	105	19c	10	180	160	180	205
10	11	185	192	305	55	11	194	194	110	35a	10	130	138	180	165
13	7	174	190	215	68	6	190	192	95	21c	7	170	186	180	205
9	10	170	184	150						Group A ↑					
20	5	180	180	205	2	12	190	190	115						
					33	9	212	190	100						
27c	11	178	192	245	13b	8	210	190	120						
					16b	10	168	190	100						
Group B ↑															
					65	7	210	186	110						
					54	12	198	180	125						
					3b	11	202	180	130						
					10b	12	210	180	80						
Ave. B.	8.8	177	187	224	Ave. B.	9.8	197	187	108						
Ave. AB.	8.0	190	205	247	Ave. AB.	8.8	195	199	106	Ave. A.	9.3	167	174	190	215

TABLE 1—*Concluded*

NON-LACTATING (25), RESTING RATS					LACTATING (39) RATS					NON-LACTATING, EXERCISED (31) RATS					
Rat number	Litter number	Body weight before pregnancy	Body weight at 10 days	Weight of uterus	Rat number	Litter number	Body weight before pregnancy	Body weight at 10 days	Weight of uterus	Rat number	Liater number	Body weight before pregnancy	Body weight after delivery	Body weight at 10 days	Weight of uterus
		gm.	*gm.*	*mgm.*			*gm.*	*gm.*	*mgm.*			*gm.*	*gm.*	*gm.*	*mgm.*
14a	11	160	176	300	45	8	194	178	80	5c	8	140	142	178	235
17a	14	160	176	230	12b	9	188	174	115	2b	12	140	188	178	205
18c	9	170	172	260	13a	12	160	174	100	4c	11	140	160	174	265
7a	—	172	170	240	47	5	170	170	100	42a	11	136	162	172	150
16a	6	140	170	245	20b	2	174	170	125	20a	—	130	173	170	160
19b	7	156	162	220	23b	5	182	162	100	30a	7	132	174	170	235
16	8	160	160	190	18b	4	176	160	80	11c	10	135	160	170	220
18a	11	140	160	220	17b	8	150	160	95	22c	7	156	158	170	160
10a	9	165	160	215	46	9	188	160	85	46a	5	130	146	168	185
					12a	10	144	160	95	27a	9	154	170	166	200
Group C ↑					Ave. C.	7.2	173	167	97	29c	11	170	164	164	190
Ave. C.	8.1	158	167	235						40a	5	136	156	164	200
Group D					34a	8	170	154	75	3c	9	150	168	160	175
					9a	4	130	150	100	23c	9	168	158	160	200
					22b	7	180	150	75	26c	4	166	160	160	165
					8a	10	120	146	105						
					11b	8	180	144	75						
					6b	8	160	140	135	Group B ↑					
					Ave. D.	7.5	156	147	94	Ave. B.	8.4	145	162	168	196
					Ave. CD.	7.3	167	160	96						
Ave. all	8.4	178	191	243	Ave. all	8.2	183	183	102	Ave. all	8.9	156	168	180	206

The non-lactating rats gained an average of 7.8 per cent, approximately, in body weight during pergnancy and lactation. The lactating rats gained an average of 3.4 per cent during pregnancy and lost this gain during lactation. The exercise group gained an average of 9 per cent, approximately, during pregnancy and 15 per cent during pregnancy and lactation.

post-partum period gained 7.8 per cent in body weight, while the group of rats that nursed young showed no gain. However, the lactating rats during pregnancy made an average gain of 3.4 per cent, which gain was lost as a result of lactation, the loss and gain in weight ranging between 9 and 15 per cent respectively. This variation in body weight incident to lactation caused us to average the weights of the uteri of the lactating rats that either lost, gained or showed no change in body weight during lactation. The results were: the average weight of the uterus of the rats that gained body weight during the lactation period was 101 mgm.; of the rats that lost, 101 mgm.; of the rats showing no change, 100 mgm. This showed that the change in body weight during the lactation period had no appreciable effect on the rate of involution of the uterus. The average weight of the uteri of the lactating rats that gained during both pregnancy and lactation was 105 mgm.; of those that lost, 100 mgm.; of those that showed no change, 101 mgm. Although we doubt that this difference is significant, the trend is in the direction that might be anticipated over a growth period of thirty-seven days (pregnancy plus lactation) (15), and coincides with our calculations that the uterine body weight ratios of all the rats in the different body weight groups correlates better when the rats are arranged on the basis of the final rather than the initial body weight.

No satisfactory correlation could be made between litter size and the rate of involution of the uterus. This argues against nitrogen demand as an important factor in the rate of involution.

None of the lactating rats manifested an oestrous cycle prior to the 16 day period. This effect of lactation is well known (13, 14). All of the non-lactating rats manifested an oestrous cycle prior to 16 days and exercise did not appreciably delay the post-partum onset of oestrus (average time of onset of oestrus in non-lactating-resting group, 6.9 days; in the non-lactating-exercise group, 6.6 days).

The effect of exercise. The effect of the two ten-minute daily periods of rather vigorous exercise on the rate of involution of the non-lactating rat is not nearly as definite as the effect of lactation. The data on 31 rats are given in table 1. It is to be noted that the exercised group gained considerable body weight (average 9 per cent) during pregnancy, and continued to gain (average, 6 per cent additional) during the post-partum exercise period. In fact, this group gained more than the non-lactating-resting group. This may have been due to the smaller rats in the exercise group; but it is quite evident that the exercise did not affect the post-partum weight as did lactation.

Because the difference between the mean weight of the uteri of the non-lactating-resting rats and that of the non-lactating-exercised rats was relatively small in contrast to the lactating rats, the probable error of the

differences between the means had to be determined. The essential data are given in table 2. In group I (table 2) the difference between the mean of all the non-lactating-resting rats and the mean of 16 non-lactating-exercised rats selected so as to yield a similar final weight is 28 (±8.3), which is a significant difference. The difference between the means, in group II, the method of comparison being reversed, is 22 (±7.82), which is also significant, but not quite so definite. In group III the data are compared on the basis of the initial rather than the final body weights. As might be expected the difference, 18 (±9.24), is not so

TABLE 2

	GROUP	LITTER AVERAGE NUMBER	AVERAGE INITIAL BODY WEIGHT	AVERAGE FINAL BODY WEIGHT	AVERAGE UTERINE WEIGHT	DIFFERENCE WITH P.E. OF DIFFERENCE IN THE MEANS
			grams	*grams*	*mgm.*	
I	All of non-lactating, resting rats (25)	8.4	178	191	243	28 (±8.3)
	16 non-lactating, exercised rats: Group A, table 1	9.3	167	190	215	11.5%
II	18 non-lactating, resting rats, including rats 31, 43, 11, 60 in group A, table 1, and rats in groups B and C	8.3	170	181	228	22 (±7.82)
	All of non-lactating, exercised (31) rats, table 1, groups A and B	8.9	156	180	206	10.6%
III	A selected group of non-lactating, resting rats (13), selected so as to yield an initial body weight of 171 gm. Initial body weight 185–156 gm. Final body weight 212–160 gm.	8.7	171	180	231	18 (±9.24) 7.7%
	A selected group of non-lactating, exercised rats (15), selected so as to yield an initial body weight of 171 gm. Initial body weight 190–156 gm. Final body weight 204–164 gm.	9.0	171	187	213	

significant; yet, the difference again shows that exercise had some effect in increasing the rate of involution of the uterus. Expressed in percentage, we believe that data show that the exercise increased the rate of involution 11 per cent, which is considerably less than the 58 per cent increase caused by lactation.

Our observations on the effect of pregnancy and lactation on the body weight of the rat in general compare favorably to those of Simmonds (16), whose data show that the average body weight remains stationary (range about −15 to +50 per cent; our range, −9 to +15 per cent).

Discussion. Our observations on the effect of lactation on the rate

of involution of the uterus confirm by a different method those of Kuramitsu and Loeb (13). Our method, we believe, permits a more satisfactory quantitation.

Three factors should be thought of in considering the effect of lactation on the rate of involution of the uterus: One, a nitrogen demand resulting from lactation; two, the mammo-uterine reflex which causes contraction of the uterus, and hence improvement in blood supply and lymph drainage and in turn facilitates the natural tendency of the post-partum uterus to involute; and third, endocrine factors. In regard to the first factor, our evidence indicates that on an ad libitum diet the factor of nitrogen demand is not a chief factor. This is indicated by the failure of the decrease in uterine weight to correlate satisfactorily in the lactating rats whether they gained, lost or manifested no change in body weight during the lactation period. The relatively slight effect of exercise may have been due to such a factor; in fact that is one reason why we were interested in determining the effect of exercise and were surprised in regard to our findings. Yet, the effect of exercise may have been due to stimulation of circulation and possibly to some reflex stimulation of uterine activity. In regard to this factor the effect of thyroid feeding and a submaintenance, low protein diet should be studied. We believe the second factor, the mammo-uterine reflex, to be very important. Such a reflex has been definitely demonstrated in monkeys by Ivy, Hartman and Koff (17). The endocrine factor is undoubtedly very important. Lactation delays the onset of the ovarian-uterine cycle as revealed by the vaginal changes. The ovarian-uterine cycle was not appreciably delayed by the exercise, which in addition to the absence of the mammo-uterine reflex explains why exercise exerted relatively little effect on the rate of involution. That the effect of the ovaries is marked is shown by the results of Kuramitsu and Loeb (13). The effect of castration after parturition, however, is not as great as the effect of lactation, since nursing still exerts a slight effect on involution after castration. In fact, Kuramitsu and Loeb conclude that probably the changes resulting in the uterus after castration render difficult the realization of the effects of either the lack of nursing or of nursing. Since oestrin increases the irritability of the uterine musculature (and promotes vascularity), a uterus under the influence of some oestrin would be more responsive to the mammo-uterine reflex effects.

SUMMARY AND CONCLUSIONS

1. When the uteri of non-lactating (25) and lactating rats (39), receiving a standard diet ad libitum, were weighed sixteen days after parturition, it was found that the mean weight of the latter group was 58 per cent less than that of the former group. Thus, lactation increases the rate of involution of the uterus very decidedly.

2. When non-lactating rats (31) were exercised daily for sixteen days after parturition, the mean weight of the uteri was decreased only by 11 per cent as compared with non-lactating-resting controls. Thus, moderately vigorous exercise only slightly augments the rate of involution of the uterus.

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THE CROSSED RESPIRATORY IMPULSES TO THE PHRENIC

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Langendorff (1887) and Girard (1890) observed contractions of one half of the diaphragm in rabbits and dogs after an ipsilateral semisection of the spinal cord above C_3 and severance of the contralateral phrenic nerve. Schiff (1894) and Porter (1895) showed that the crossing of the respiratory impulses did not occur until the phrenic was cut on the opposite side. Porter further demonstrated that this crossing occurred at the level of the phrenic nuclei, not above or below.

We have failed to find any reports of further work on this phenomenon since Porter's observations. All the explanations which have been offered to account for it seem to contradict some known properties of nerve impulses or to postulate new, unparalleled properties of the nerve cells. Thus, Schiff (1894) spoke of the section of a phrenic acting as a "specific enhancing agent" of the activity of the opposite phrenic. He laid great stress on asphyxia, but did not mention the possible blocking of afferent impulses by the section. Porter (1895) suggested that some dendrites of the phrenic motoneurones cross over; the impulses they carry are not usually sufficient to cause a contraction of the opposite side of the diaphragm; but, after section of the phrenic nerve a greater portion, perhaps the whole of the "descending impulse" of that side, passes through the crossed dendrites into the phrenic cells of the opposite side. Similarly, Barcroft (1934) speaks of the impulse coming down the side where the phrenic is cut being "thwarted" along its usual path, therefore "pushing" across to the opposite phrenic along which it discharges.

The present study was undertaken with the purpose of obtaining, if possible, a satisfactory explanation of the "crossed phrenic phenomenon." We felt that before new properties are postulated for nerve cells or synapses all the possibilities of explaining the data in terms of the known properties should be exhausted. The experiments quoted did not control for the possible rôle of afferent nerve impulses and asphyxia.

METHOD. With dial (Ciba) anesthesia at the doses (per kgm.) bracketed, the following animals were used: monkeys (0.5 cc.), dogs (0.7 cc.),

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cats (0.75 cc.), guinea pigs (0.5 cc.), rabbits (0.55 cc.) and woodchucks (0.6 cc.). The dial was injected intraperitoneally and supplemented with ether or urethane whenever necessary.

The vagi and phrenics were approached in the neck. For stimulation of the cut nerves, buried shielded electrodes were employed, delivering shocks from a Harvard induction coil with 5 volts in the primary circuit. Reversible blocks of the phrenics were obtained by applying pledgets of cotton soaked with ether to the dissected nerves at the base of the neck until paralysis of the corresponding hemidiaphragm was complete. The sections of the spinal cord and dorsal roots were all performed acutely. These sections were verified macroscopically at autopsy.

A tracheal cannula was inserted for artificial respiration, whenever

TABLE 1

	MONKEYS	DOGS	CATS	GUINEA PIGS	RABBITS	WOODCHUCKS
Respiratory hemiplegia on spinal semisection	Yes	Yes	Sometimes	Yes	Yes	Yes
Crossed diaphragmatic contractions on cutting the vagi, cervical sympathetics and depressors	No	Yes	No	No	No	No
Crossed diaphragmatic contractions with asphyxia (see text for exceptions)	No	No	No	No	No	?
Crossed diaphragmatic contractions on cutting the active phrenic	No	Yes	Yes	No	Yes	Yes
Crossed costal movements	No	No	No	No	No	No
Unilateral costal respiration adequate	Yes	Yes	Yes	No	No	?
Unilateral diaphragmatic respiration adequate	Yes	No	Yes	Yes	Yes	Yes

necessary, and to produce asphyxia, either by closing the cannula or by connecting it to a rubber balloon containing nitrogen or expired air.

The movements of both halves of the diaphragm were recorded as follows. A midline abdominal incision was made from the umbilicus to the base of the xiphoid cartilage. Two long clamps were placed on the abdominal walls, immediately below the ribs. Sometimes a third clamp was fixed to the xiphoid cartilage. The clamps were lifted by a horizontal rod so that the diaphragmatic region of the animals was slightly raised from the animal board. This procedure was found not to interfere with respiration. After the abdominal viscera were pressed caudad with cotton, which also served to protect them from unnecessary exposure, the anterior half or more of the diaphragm was accessible and visible. Serrefines were then placed symmetrically toward the center of each dome

and connected, via pulleys, to the recording levers. Downward excursions of these denote, therefore, inspiration.

This procedure was adopted for recording because it was deemed of importance to be able to observe the diaphragm directly, in order to distinguish clearly active from passive movements. Other respiratory movements such as the costal, abdominal, laryngeal and nasal, were also observed directly.

RESULTS. A. *Monkeys.* A spider monkey and three macaques were studied. In three of them a semisection of the spinal cord at C_2 totally paralyzed all the thoracic respiratory movements on the same side; the

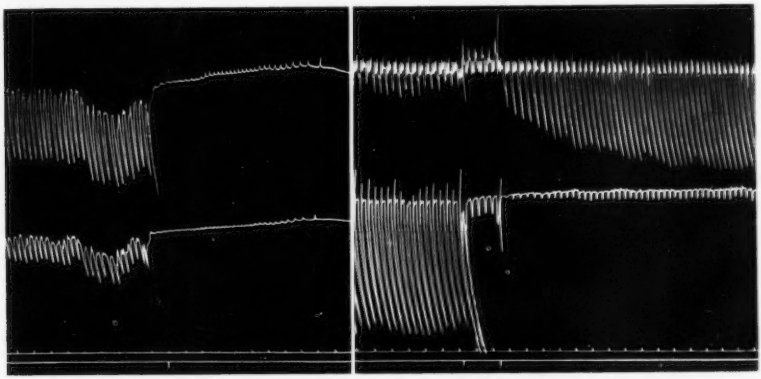

Fig. 1 Fig. 2

Fig. 1. Macaque monkey. Left spinal semisection at C_2; complete transection at C_7. Vago-sympathetics cut. Upper record: right, and lower record: left half of the diaphragm. At lower signal, section of the right phrenic. The movements occurring after this section were transmitted from the neck and head muscles. In this and the succeeding figures the upper signal records 5 second intervals.

Fig. 2. Dog. Left spinal semisection at C_2; complete transection at C_7. Upper record: left, and lower record: right half of the diaphragm. Between the lower signals, section of the right phrenic.

fourth monkey will be described separately. In one of the macaques a complete transection of the cord was also made at C_7, eliminating all costal respiration. Cutting the vago-sympathetic trunks (two animals) induced only slight typical effects on the active, but no change on the paralyzed side. Section of the discharging phrenic failed in the three animals to elicit respiratory movements of the semisected side. The animal which had had the complete transection at C_7 died of asphyxia, after the larynx and nose had shown the movements which correspond to labored breathing and to gasps (fig. 1). The other two monkeys survived the section of the phrenic, the costal breathing of the active side sufficing

to maintain an adequate ventilation; they were sacrificed 40 and 60 minutes later, respectively, without having shown any further diaphragmatic contraction.

In the fourth monkey, a macaque, a left spinal semisection at C_3 failed to paralyze the ipsilateral diaphragmatic contractions, although they were smaller than those of the other hemidiaphragm. An ether block (see p. 502) was applied to the right phrenic, whereupon the entire diaphragm was paralyzed. On recovery from this block the left phrenic was cut, but the contractions of the left hemidiaphragm, which had now reappeared, were not abolished by this section. Cutting the right phrenic permanently paralyzed all the diaphragm. Obviously the contractions of the left hemidiaphragm occurred in response to nerve impulses delivered by the right phrenic, a case of peripheral crossing. With the possible

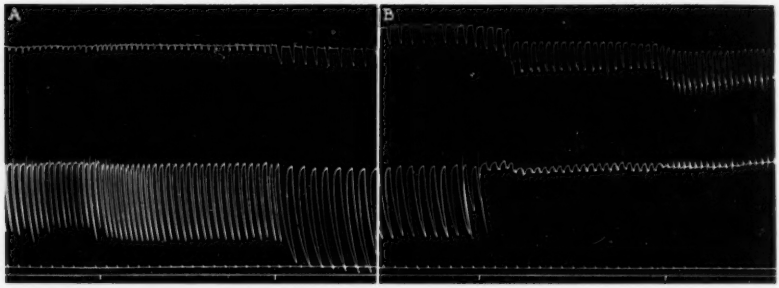

Fig. 3. Dog. Right spinal semisection at C_2; complete transection at C_7. Upper record: right, and lower record: left half of the diaphragm.

A. Section of the left, and then the right vago-sympathetic nerves.

B. Section of a branch, then of the remainder of the left phrenic.

exception of some cats (see section C), this was the only unquestionable instance of a peripheral crossing of the phrenic nerve supply encountered in all the animals studied. Since the ether block resulted in total paralysis of the diaphragm we may conclude that in this monkey, as in the other three, the crossed phrenic phenomenon did not occur.

B. *Dogs.* A spinal semisection at C_2 and a complete transection at C_7 were performed on 6 dogs; in 5 others only the semisection at C_2 was made. In all cases the diaphragm and the costal muscles were paralyzed on the side of the semisection. Cutting the phrenic on the opposite side, with the vagi intact, promptly resulted in respiratory movements of the previously paralyzed half of the diaphragm in one animal out of 3 (fig. 2). The two other dogs also belonged to the group which had a spinal transection at C_7. Cutting the active phrenic in these two aminals led to immediate asphyxia; artificial respiration and severance of the vagi suc-

ceeded in bringing about adequate diaphragmatic contractions in the opposite half of the diaphragm in only one of them.

In the remaining 8 dogs the vago-sympathetic nerves were cut before section of the phrenic. With one exception, these cuts immediately led to the sharing of the previously paralyzed hemidiaphragm in the respiratory movements (fig. 3A). Subsequent section of the originally active phrenic increased only slightly the contractions of the opposite side (fig. 3B). The exception mentioned was a dog with only a spinal semisection at C_2 in which cutting the vagi did not lead to respiration of the paralyzed

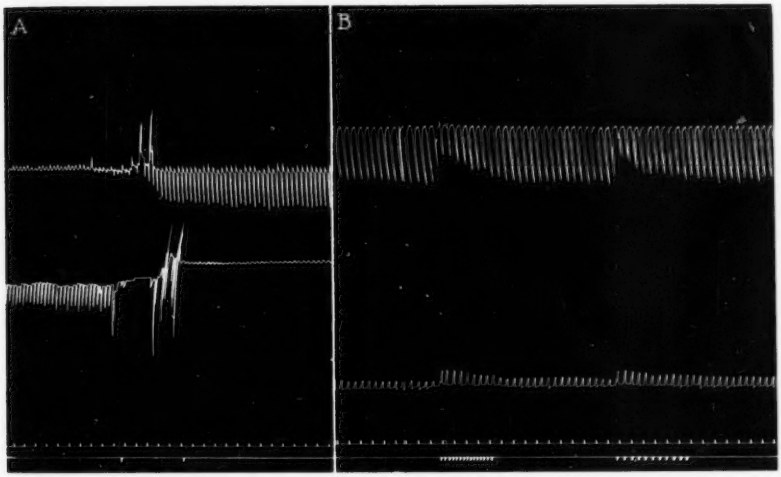

Fig. 4. Cat. Upper record: left, and lower record: right half of the diaphragm. A. Left spinal semisection at C_2. Between signals, section of the right phrenic. B. Left spinal semisection at C_2; right phrenic and vagi cut. At signals, short tetani applied to the central end of the cut phrenic; coil distance: 5 cm.

hemidiaphragm; subsequent section of the active phrenic resulted in slight diaphragmatic contractions on the opposite side.

In general, diaphragmatic respiration without costal participation—i.e., in the dogs with complete spinal transection at C_7—was not sufficient for adequate ventilation of the animals. Especially deficient were the dogs in which only the hemidiaphragm on the side of the spinal semisection contracted after severance of the other phrenic; they only survived for short periods.

Afferent stimulation of the cut phrenic resulted in increased and more frequent respirations in 4 out of the 5 animals in which it was tested; in the other dog slight inhibitions were observed. Strong stimuli (coil distance 7 cm. or less) were necessary to elicit these effects.

Asphyxia was produced in 4 animals by occluding the tracheal cannula during 15 to 30 seconds before severing the vagi and the phrenic. It did not result in contractions of the paralyzed hemidiaphragm.

C. *Cats.* Eight animals were studied. In all of them a spinal semisection was performed at C_2 or C_3. This semisection resulted in clear ipsilateral diaphragmatic paralysis in 3 cats only; in the other 5, movements of both hemidiaphragms were recorded, although the autopsy showed a complete semisection in at least 2 of them. The passive movements of a paralyzed hemidiaphragm (e.g., after section of a phrenic) are, however, as a rule very marked in cats. No thorough controls were made to rule out entirely such passive movements or the possibility of a peripheral phrenic crossing (see section A) in these 5 cats. We shall therefore report in detail only the other 3, in which the respiratory hemiplegia was clear.

Section of the vagi and cervical sympathetics did not produce any crossed phrenic effects. Asphyxia was likewise inefficient in this respect. Section of the active phrenic, on the other hand, resulted in crossed diaphragmatic contractions (fig. 4A). The crossing, however, did not occur promptly (cf. section E), but only appeared after a delay of from 10 to 60 seconds. The contractions of the crossed side were quite small, as compared with those of the other side before the section of the phrenic.

The detailed protocol of one of the 3 cats which showed a respiratory hemiplegia is the following. The spinal cord was semisected on the left side between C_2 and C_3, and completely transected between C_5 and C_6. The dorsal roots C_3, C_4 and C_5 were cut on both sides. The cranial nerves IX, X, XI and XII were cut on both sides. The cervical sympathetics were also cut. The right hemidiaphragm was breathing. The right phrenic was then severed, whereupon, after a delay of about 10 seconds the left hemidiaphragm started contracting. The ventilation was not sufficient, however, for the needs of the animal; artificial respiration was given; when after 10 minutes this was stopped the left hemidiaphragm again showed respiratory movements, then gasps, and the cat died.

Afferent stimulation of the cut phrenic nerves (4 animals) inhibited the respiratory excursions of the other side (fig. 4B). This inhibition was only obtained, however, with relatively strong stimuli (coil distance 6 cm. or less), weaker shocks producing no effects.

D. *Guinea pigs.* Three animals were tested. In two of them a right spinal semisection was made at C_2. A typical respiratory hemiplegia ensued. The left phrenic was then cut; no crossed effect was obtained and the diaphragm was fully paralyzed. The left costal respiration persisted but was incapable of maintaining life; the animals died in a few minutes.

In the third guinea pig, besides the right spinal semisection at C_2, a

complete transection at C_7 was made, the dorsal roots C_3, C_4, C_5 and C_6 were cut on both sides and the vagi were severed. The left hemidiaphragm was contracting. Section of the left phrenic resulted in quick death. No contractions of the right hemidiaphragm were observed, although nasal and laryngeal respiratory movements were apparent until the end.

E. *Rabbits.* Observations were made on 25 animals. In all of them the spinal cord was semisected at C_2, producing a respiratory hemiplegia. In 4 a complete transection was also made at C_6 or C_7, paralyzing all costal respiration; the breathing movements of one hemidiaphragm sufficed to keep the animals alive.

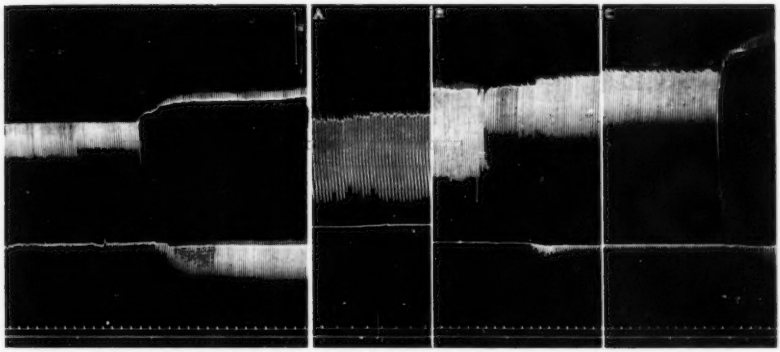

Fig. 5

Fig. 6

Fig. 5. Rabbit. Left spinal semisection at C_2. Upper record: right, and lower record: left half of the diaphragm. Vagi cut. At signals, right phrenic cut, first below C_4, then below C_6.

Fig. 6. Rabbit. Left spinal semisection at C_2. Upper record: right, and lower record: left half of the diaphragm.

A. Section of the right 6th cervical nerve.

B. Section of the right 5th cervical nerve.

C. Section of the right 3rd, then 4th cervical nerves.

Section of the vagi (13 animals), cervical sympathetics and depressors (8 animals) and carotid-sinus nerves (4 animals) failed to produce a crossed diaphragmatic activity.

Section of the active phrenic, whether the vagi were intact (6 animals) or severed (9 animals), but with uncut cervical sympathetics, depressors and carotid-sinus nerves, almost immediately resulted in respiration of the opposite, previously paralyzed hemidiaphragm (figs. 5 and 6). When the entire phrenic was not cut at one stroke, but its several roots were severed successively, the crossing over only occurred when the last root was cut, although paralysis of a considerable portion of the diaphragm resulted from the first sections (cf. fig. 2). Schiff (1894) reported that the

crossing only occurred when the component of the phrenic contributed by C_6 was cut. We failed to confirm this statement, for if the cervical nerves C_3, C_4, C_5 and C_6 were cut successively at their emergence from the spinal column, the crossing occurred when C_5 or C_6 was cut if the sections were made in the order stated, but the crossing only occurred on cutting C_4 if the sections were made in the reverse order (fig. 6).

When the whole phrenic nerve was cut at one stroke the crossing appeared immediately. Indeed, the first crossed contraction, though small, occurred usually at the precise time at which the other side would have contracted, had its nerve been intact (fig. 5). A gradual increase of the crossed responses appeared thereafter and in 10 to 20 seconds a steady state was attained.

The method adopted for recording is mainly qualitative. No quanti-

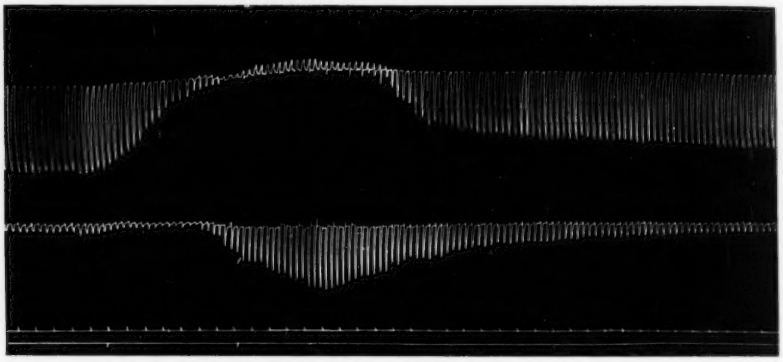

Fig. 7. Rabbit. Left spinal semisection at C_2. Upper record: right, and lower record: left half of the diaphragm. Vagi cut. Between signals, application of a pledget of cotton soaked with ether to the right phrenic.

tative judgments can be made from the records, therefore, as regards the magnitude of the contractions on the crossed as compared to the opposite hemidiaphragm. It was observed, however, that the costal excursions increased after section of the phrenic, in the animals in which they had not been abolished by a complete spinal transection (cf. fig. 6). Furthermore, no contractions of the crossed slip of the diaphragm inserting at the xiphoid cartilage were detected. It may be concluded, therefore, that the excursions of the crossed hemidiaphragm were not as extensive as those of the originally active side.

Reversible crossed phrenic discharges were readily obtained by applying for short periods pledgets of cotton soaked with ether to the phrenic opposite the spinal semisection, instead of cutting. Figure 7 illustrates a typical example. As many as 7 crossings were obtained in the same

animal by this procedure. The results differed from those elicited by the section of the phrenic as follows. Whether the paralysis of the active hemidiaphragm occurred rapidly (5 to 10 seconds) or very gradually (several minutes), by applying various amounts of ether, the crossed contractions did not appear until practically complete cessation of respiratory activity in the blocked side. Indeed, it was possible by removing the ether opportunely to paralyze almost entirely the diaphragm without the appearance of any crossed contractions.

In contrast with the practical lack of simultaneous contractions of the two sides at the onset of the ether block, during the recovery the direct side usually started contracting before the crossed activity had subsided,

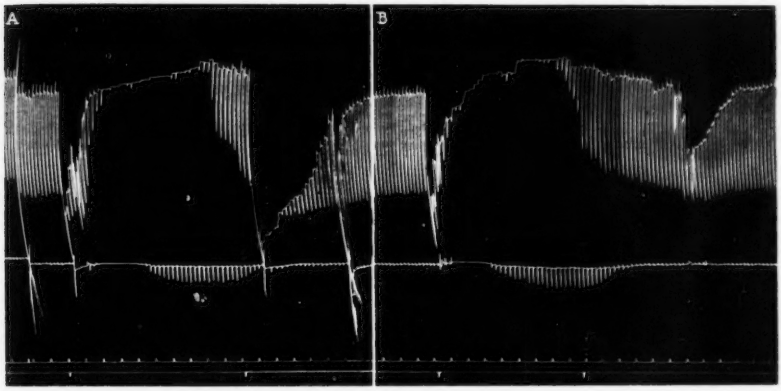

Fig. 8. Rabbit. Left spinal semisection at C_2. Right C_6 nerve cut at its emergence from the spinal column. Upper record: right, and lower record: left half of the diaphragm. Between the signals, direct current applied to the right phrenic between C_5 and C_6.

A. Anode cephalad.

B. Cathode cephalad.

so that simultaneous movements were sometimes present for as long as several minutes.

Reversible blocks were also obtained by direct current as follows. The 6th cervical nerve was cut at its emergence from the spinal canal, and a pair of shielded electrodes was placed on the uncut active phrenic between C_5 and C_6. The direct current was drawn from a battery connected to these electrodes through a potentiometer. The intensity was gradually increased until complete paralysis occurred. The results were in all points identical with those obtained from the ether blocks, previously described. Reversing the current did not modify these results—i.e., it was immaterial for the crossing whether the anode or the cathode was applied cephalad (fig. 8).

In no instance, whether after section of the phrenic or during the ether or direct current blocks, was crossed costal respiration observed.

In 4 rabbits, in which both vagi, cervical sympathetics and depressors were cut before severing the active phrenic or blocking it by ether, the latter procedures did not result in crossed diaphragmatic contractions (fig. 9). In 4 other rabbits, on the other hand, an ether block was applied after severance of the vagi only, with the typical crossed effects; when the cervical sympathetics, depressors and carotid-sinus nerves were subsequently cut, the ether block now was effective in eliciting crossed contractions. These contractions, however, were delayed and smaller after than before severance of the circulatory proprioceptors. An explanation for these apparently inconsistent results will be suggested in the discussion.

Asphyxia was produced by either occlusion of the tracheal cannula

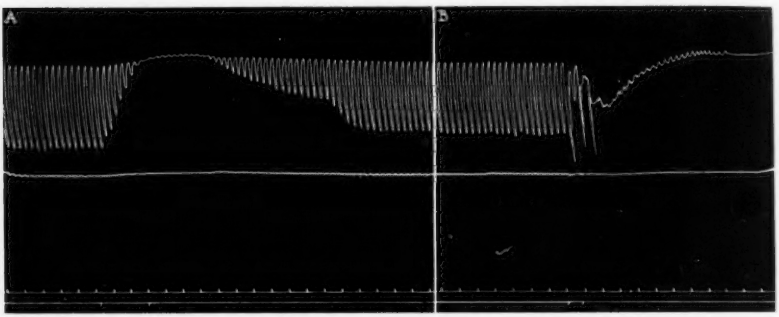

Fig. 9. Rabbit. Left spinal semisection at C_2. Upper record: right, and lower record: left half of the diaphragm. Vagi, cervical sympathetics, depressors and carotid-sinus nerves cut.

A. Between signals, cotton soaked with ether applied to the right phrenic.

B. Section of the right phrenic.

or connection of it with a rubber balloon containing expired air or nitrogen. Neither procedure elicited crossed diaphragmatic contractions when performed before cutting the active phrenic or blocking it with ether (fig. 10), even if the asphyxia was carried till failure of the respiration. On the contrary, asphyxia did sometimes lead to crossed contractions when applied after an ether block had already elicited responses from the crossed side; these positive results were only obtained when the depressors or carotid-sinus nerves were intact (fig. 11).

Further tests for the rôle of asphyxia in the production of the crossed phenomenon were the following. In 3 rabbits artificial respiration was administered and the thorax was widely opened before section or block of the active phrenic. The ventilation was adjusted to very slightly less than that which would cause apnea, and was kept constant thereafter.

Section of the phrenic again evoked crossed diaphragmatic activity. In 3 other rabbits the phrenic nerves were dissected on both sides between C_5 and C_6 after the spinal semisection at C_2. The action potentials on the active side were amplified and led to a loud speaker and a cathode-ray oscillograph, so that they could be heard and photographed. Curare was then injected in a dose sufficient to paralyze all respiratory movements. Artificial respiration was administered, and adjusted as before so that nerve impulses were rhythmically discharged through the phrenic on the direct side. The crossed phrenic was still totally inactive in these

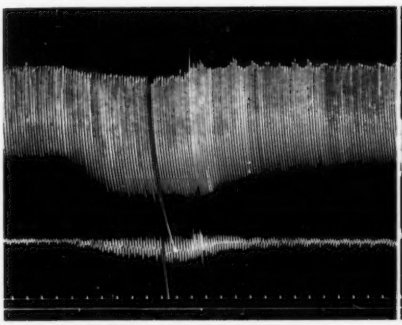

Fig. 10

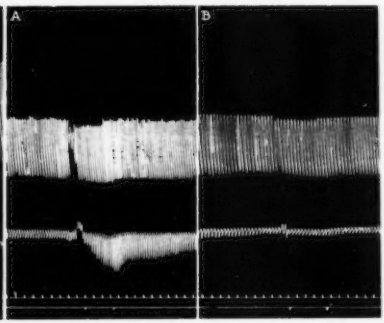

Fig. 11

Fig. 10. Rabbit. Left spinal semisection at C_2. Upper record: right, and lower record: left half of the diaphragm. Between signals, rubber balloon containing expired air connected to the tracheal cannula. The movements of the left hemidiaphragm were all unquestionably passive.

Fig. 11. Rabbit. Left spinal semisection at C_2. Upper record: right, and lower record: left half of the diaphragm. Six reversible crossings were obtained by means of ether blocks in the course of the experiment before these records were made. Vagi, cervical sympathetics and depressors cut.

A. Between signals, rubber balloon with nitrogen connected to the tracheal cannula.

B. Three minutes after A. Carotid-sinus nerves cut in the interval. Between signals, same as A.

conditions. Turning off the artificial respiration for 20 to 30 seconds merely intensified the discharges in the direct side, but did not produce any crossed activity. Severance of the active phrenic below the electrodes, with constant artificial respiration, led to bilateral simultaneous discharges—i.e., the crossing occurred and the nerve impulses on the side originally active were not apparently modified. The discharges on the crossed side were not as intense as on the phrenic directly connected to the medulla.

Intermittent peripheral stimulation of the cut phrenic did not inhibit the crossed respiratory impulses (fig. 12C) unless marked hyperventilation

was produced, and consequent apnea. When this hyperventilation was prevented by letting the animal breathe into a rubber balloon with expired air, no inhibition could be elicited.

Strong afferent stimulation of the cut phrenic between C_5 and C_6 was usually without influence on the respiratory activity of the opposite hemidiaphragm; in only two instances out of seven did a questionable inhibition appear. The possibility of afferent fibers in the phrenic playing a rôle in the appearance of the crossed phrenic phenomenon was further tested by severance of the dorsal roots as follows. In one rabbit a left spinal semisection was made between C_2 and C_3 and the right dorsal

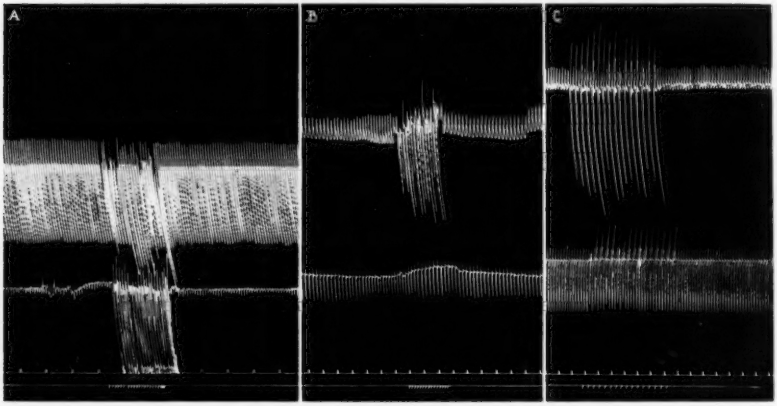

Fig. 12. Rabbit. Left spinal semisection at C_2. Vagi cut. Upper record: right, and lower record: left half of the diaphragm.

A. At signals short tetani applied directly to the left hemidiaphragm.

B. After cutting the right phrenic. At signals short tetani applied directly to the right hemidiaphragm. The movements of this hemidiaphragm not produced by the stimuli are passive upward excursions produced by the activity of the ipsilateral costal respiration.

C. Short tetani applied to the peripheral end of the cut right phrenic.

roots C_3, C_4, C_5 and C_6 were cut. A complete spinal transection between C_6 and C_7 was also performed. Only the right hemidiaphragm was contracting. Section of the right phrenic immediately resulted in paralysis of the right and respiration of the left hemidiaphragm. In another rabbit a similar preparation was made, but in addition the vagi were cut before severing the phrenic. Asphyxia was tested, and failed to produce contractions of the paralyzed hemidiaphragm, while section of the phrenic elicited typical results.

Other tests for afferents in the phrenic nerves were made by stimulating intermittently the paralyzed hemidiaphragms while the other side was

breathing. Such a stimulation did not inhibit either the half opposite the spinal semisection, when the phrenics were intact, or the crossed activity on the side of the semisection after the opposite phrenic had been cut (fig. 12A and B).

F. *Woodchucks.* Although only two woodchucks (*marmota monax*) were successfully studied, the results are reported because they were quite clear and because of their interest in comparing them with the other species.

In one of the animals a spinal semisection was made at C_2, which resulted in an ipsilateral respiratory hemiplegia. Section of the vagi elicited only slight typical effects. Section of the active phrenic was promptly followed by crossed diaphragmatic respiration. Neither central nor peripheral stimulation of the cut phrenic with various frequencies and intensities yielded inhibition of the crossed respiratory movements.

In the other woodchuck, in addition to a spinal semisection between C_2 and C_3, a complete transection was made between C_6 and C_7 and the dorsal roots C_3, C_4, C_5 and C_6 were cut on both sides. Only the hemidiaphragm on the side opposite the spinal semisection was contracting. An ether block was applied to the active phrenic, which resulted in a reversible crossing in all points similar to those obtained in the rabbits. Subsequent section of the phrenic after recovery from the ether block led to a permanent crossing of the impulses to the diaphragm. In this woodchuck also neither peripheral nor central stimulation of the cut phrenic inhibited the crossed diaphragmatic activity.

Discussion. The differences between the several species studied are summarized in table 1. The monkeys and guinea pigs on the one hand, and the cats, rabbits and woodchucks on the other, behaved similarly. The dogs differed from all the other species. Obviously, with such heterogeneous results a phylogenetic analysis would not be fruitful. The attempt to systematize these species differences in terms of the relative importance of diaphragmatic and costal respiration, as judged from the present data, leads likewise to inconsistent conclusions (table 1). Thus the dogs and rabbits, in which crossed phrenic effects are present, have apparently opposite relative importance of the costal and diaphragmatic respirations.

The dogs stand out as the unique species in this series, in which severance of the vago-sympathetic-depressor nerves led to crossed respiratory discharges. Quantitatively the slowing and deepening of respiration consequent upon double vagotomy was also greater in the dogs than in the other species. The larger vagal regulation of the respiratory activity of dogs may perhaps be correlated with other biological features of this animal, such as its special adaptation for running. We must, however, leave the phylogenetic aspect of the problem for further investigation.

Contradictory reports have appeared in the literature concerning the question of the effects of a spinal semisection above C_3 on diaphragmatic respiration. While some observers affirm that such a semisection leads to a hemiparesis, others state that both halves of the diaphragm may continue breathing (see Cordier and Heymans, 1935, for references). Indeed, this persistence of contractions on the semisected side was once looked upon as a strong argument in favor of the existence of spinal respiratory centers.

In some species, e.g., the dogs and cats, the passive movements of a paralyzed hemidiaphragm, produced by the activity of the other hemidiaphragm and of costal respiration, are so marked that it is quite difficult, even by careful direct observation, to distinguish passive from active changes (cf. Schiff, 1894). One of the controls which Langendorff (1887) used to establish this distinction was to cut the phrenic on the side opposite the semisection, obviously a poor control in view of the knowledge acquired since. A sure method, which has not been used, however, would be to record the muscle action-potentials.

Another source of error which has usually not been duly controlled is the possibility of a peripheral crossing, such as that encountered in one monkey in the present observations (p. 498). With the exception of some of the cats (section C), on which the controls were not made to decide whether a peripheral crossing occurs or not, all the other observations made agree in showing that spinal semisection results in respiratory hemiparesis, and that in the monkeys and guinea pigs there probably is no crossed phrenic spinal path. As regards the cats, Henderson and Craigie (1936) have also recently reported respiratory hemiplegias on spinal semisections.

Crossed phrenic activity appeared in the dogs (figs. 2 and 3), the cats (fig. 4), the rabbits (figs. 5, 6, 7 and 8) and the woodchucks (section F). In the dogs, although severance of the vagi usually produced contractions of the paralyzed hemidiaphragm (fig. 3A), the fact that cutting the phrenic increased this activity (fig. 3B), and the instance in which cutting the vagi did not elicit a crossing, while section of the phrenic did (section B), indicate that there probably exist in this species phrenic spinal arrangements similar to those of the cats and rabbits. The masking vagal effects and the irregularity of the results obtained, however, make this animal unsuitable for the analysis of the phenomenon. In the cats, likewise, the results were not consistent (section C) and the effects, when present, were not very striking. For these reasons the majority of the controls were carried out in rabbits, and we shall now concern this discussion mainly with them.

When the active phrenic is cut after the spinal semisection at C_2 several effects ensue. First, there may appear a certain degree of asphyxia, particularly when the costal respiration has been eliminated by the spinal

transection at C_7. Secondly, afferent impulses in the phrenic, arising at the active hemidiaphragm, might be interrupted by the section. Finally, since the paralysis of the corresponding hemidiaphragm entails changes in the expansion of the ipsilateral lung, or a cessation of this expansion when the costal respiration was eliminated, afferent impulses arising in the lung might also be interrupted by the section. These afferent impulses would travel in the nerves distributed to the lungs—the vagus and the sympathetic.

Undoubtedly relative asphyxia after section of the phrenic must have increased the number of discharges from the respiratory center in the medulla, particularly when the depressors and carotid-sinus nerves were intact. But that these increased discharges were not sufficient to elicit the crossed phrenic discharges was shown by the following controls. Asphyxia produced before severing the phrenic failed to evoke contractions of the paralyzed hemidiaphragm (fig. 10). And when the phrenic was cut while constant artificial respiration was applied after opening the thorax or under curare (p. 505), the crossing over occurred, although the discharges of the respiratory center were not modified by the section. We should therefore consider asphyxia as a probable adjuvant factor in the production of the crossed phenomenon, but not as a sufficient cause of it. The cases in which asphyxia did result in crossed contractions, when applied after previous blocks of the phrenic by ether (fig. 11), will be discussed below.

That the crossed effects are not due to the interruption by the section of a stream of inhibitory afferent impulses in the phrenic nerve is shown as follows. The phenomenon was not elicited by section of the corresponding dorsal roots (sections C, E and F), but readily occurred thereafter on cutting the remaining ventral root fibers. The possibility might still remain that these ventral root fibers include afferents, but central stimulation of the cut phrenic failed to stop the respiration of the opposite side (sections B, C, E and F). Similarly, direct stimulation of the paralyzed hemidiaphragm homonymous to the spinal semisection before cutting the phrenic, which should activate the corresponding afferents, failed to inhibit the respiration of the active side (fig. 12B).

Afferent phrenic impulses elicited by passive movements from the paralyzed hemidiaphragm before section of the active phrenic were eliminated as a source of inhibition in the woodchuck in which the corresponding dorsal roots were cut (section F), and by the controls in which the peripheral end of the cut phrenic was stimulated (fig. 12C). Such stimulation did not inhibit the crossed respiration.

Inhibitory afferent impulses in the vagi, arising at the lungs, were eliminated by section of these nerves (sections C and E). Inhibitory impulses reaching the centers by afferents in the sympathetic nerves could not have

been at play when the spinal cord was transected at C_6 or C_7 (sections B, C, E and F). Changes in such afferent impulses were further eliminated when the phenomenon appeared while constant artificial respiration was administered either with the thorax widely opened or after curare (section E; cf. Schiff, 1894; Porter, 1895).

Indeed, the time course of the crossed effects on application of the reversible ether or direct current blocks to the active phrenic (figs. 7 and 8) is such as to exclude *per se* both asphyxia and afferent impulses as causes of the crossing. For if either or both factors were at play the crossing should be gradual, when the paralysis is also gradual, and instead, the crossed side only enters into activity after the other side is almost completely paralyzed, regardless of the time necessary for this paralysis, and if the ether block is not complete no crossing occurs (section E).

We are led, therefore, to conclude that severance or block of the phrenic motor fibers is the necessary and sufficient cause of the crossed phrenic effects under the experimental conditions adopted. It is unlikely that a persistent antidromic bombardment of the phrenic motoneurones should be produced by the section, for the peripheral end did not discharge continuously, since the diaphragm was paralyzed on that side. The positive results of the ether and direct current blocks are further evidence against such a possibility. It appears, therefore, that the phenomenon reveals a property of neurones which differs in quality from that of conducting nerve impulses.

It was stressed in the description of the results that the events during the recovery from the ether or direct current blocks differ as a rule from those at the onset of the block (section E). While at first the crossed side does not start contracting until the originally active half is almost entirely paralyzed, during the recovery from the block the originally active side commences usually to contract long before the crossed responses have subsided. Indeed, this subsidence may take a very long time, for after an ether block had disappeared the records were never quite identical with those obtained before the application of the ether. It may be concluded that these data reveal another property of the nervous system, qualitatively different from conduction of nerve impulses. The opening of the new, hitherto unused path by the block alters the properties of the neurones or synapses involved so that they now tend to remain in use for relatively long periods of time, even though the original conditions have been practically reëstablished.

This second property accounts satisfactorily for the two apparent inconsistencies observed. Asphyxia only elicited crossed contractions when a previous ether block had been applied (fig. 11)—i.e., when the crossed path had been opened at least once. Conversely, the ether block only failed to produce crossed contractions when applied for the first time after

severance of the vagi, sympathetics, depressors and (in some animals) carotid-sinus nerves (fig. 9)—i.e., when the path had not been opened before this severance—but was effective even after these nerve sections if the path had been originally opened by an early block.[2]

Any explanation of the crossed effects which postulates a general property of the central nervous system, without consideration of special paths, such as that of Barcroft, quoted in the introduction, should be rejected, for the phenomenon is not present in all species (table 1). Schiff's (1894) statement, that the section of one phrenic specifically enhances the activity of the opposite phrenic, is really descriptive, not explanatory. Porter's interpretation (see introduction) clearly violates the all-or-none nature of nerve impulses and is not supported by observations such as those of Gasser and Newcomer (1922), Adrian and Bronk (1928), and our own (p. 505), which demonstrate that the cut phrenic does not cease to conduct respiratory impulses.

In attempting to formulate a working hypothesis for the properties of neurone paths which the data reveal, the following considerations are pertinent. An hypothesis involving only unineuronal changes from the section or block will be preferable because simpler than one postulating influences on other neurones than those cut. It does not appear plausible, furthermore, that such changes of other neurones should occur without some causal change in the cut motoneurones. The assumption has been made (Ramón y Cajal, 1909; Eccles and Sherrington, 1931; Lorente de Nó, 1935) that neurones are not polarized as regards conduction of nerve impulses—i.e., that an impulse set up anywhere will travel throughout the cell, including the axon, perikaryon and dendrites. There is no direct evidence, however, for such an assumption. Most of the experiments which demonstrate bidirectional conduction have been made on axons; the properties of typical dendrites are very little known.

To account for the phenomena under discussion in terms of nerve impulses transmitted by the cut motoneurones we must postulate either *a*, that some nerve impulses, discharged continuously from the phrenic motoneurones on the intact or direct side to the crossed phrenic nuclei (e.g., by a branch of the corresponding axons) increase in magnitude after the section of the phrenic (e.g., as a consequence of electrotonus); or we may assume *b*, that some branches, axonic or dendritic, of the motoneurones are not accessible to cellulifugal nerve impulses until the phrenic is cut or otherwise blocked.

The suggestion *a* does not seem probable for the following reasons.

[2] Experiments performed since this paper was sent to press demonstrate that a crossing may occur on cutting the active phrenic after severance of the vagi, sympathetics and depressors, even if no previous ether block was applied. The reason for the lack of crossing in the four rabbits reported here remains obscure.

Although a section of the motor fibers in the phrenic might change the magnitude of the nerve impulses through an electrotonic effect due to the injury potential, anesthetic blocks influence only negligibly the resting potential of nerve fibers (Bishop, 1932). Furthermore, the fact that reversing the direct current blocks (fig. 8) did not change the results argues against an explanation based on electrotonus. The direct evidence available does not support the suggestion, either, for Adrian and Bronk (1928) did not find any significant change in the action potentials recorded from the phrenic before and after peripheral section.

We might then assume, according to suggestion *b*, that the dendrites of the phrenic motoneurones have normally only unidirectional conduction (Gad, 1884), and that blocking the axon makes them by some unknown means capable of cellulifugal conduction, whereupon they can activate the opposite phrenic nuclei, with which they would be assumed to have synaptic connections. That dendrites may activate other neurones was a view first defended, later rejected by Ramón y Cajal (loc. cit.), but the question may still be considered open, for the evidence against such synapses is only negative.

Many other working hypotheses are possible, which would not be based on conduction of nerve impulses by the cut phrenic motoneurones. With the data on hand they would be purely speculative. The one developed is suggested only because it appears to be the simplest. On the basis of such an interpretation the data cast doubt on the assumption that nerve impulses spread normally throughout all parts of the neurones; if they did they should be active before the block or section of the phrenic; furthermore, if they did the antidromic impulses started on central stimulation of the cut nerves should activate the crossed phrenic nucleus, but they do not (sections E and F). It is of course possible, however, that this relatively simple hypothesis will prove inadequate and that changes in other neurones than those cut or blocked do occur—i.e., a change in "potential," chemical, electrical, or other, in the blocked motoneurones, might permit neighboring dendrites from the opposite side to be activated during the block.

The data obtained under curare (p. 505) lead to the conclusion that for the phenomenon to occur it is necessary to block the axons. A neuromuscular barrier such as that effected by curare is not sufficient to produce the crossing.

The two properties of the nervous system evidenced by the data—the opening of an unused path and the tendency for this path to remain open for relatively long periods—are properties which also appear in other unsolved problems of central nervous functions: the establishment of conditioned reflexes and the persistence of learned patterns. Further analysis will determine whether the similarity is meaningful.

SUMMARY

In animals of several species, under dial anesthesia, spinal semisections at C_2 produced ipsilateral respiratory hemiplegias. Section of the active phrenic led to the prompt appearance of crossed diaphragmatic activity in dogs (section B), cats (section C), rabbits (section E) and woodchucks (section F). No crossed contractions occurred in monkeys (section A) and guinea pigs (section D).

Reversible blocks of the phrenic were obtained by means of ether or direct currents. These gave reversible transient crossed contractions similar to those resulting from the sections (figs. 7 and 8; section F).

The crossed effects in the rabbits, cats and woodchucks are not due to asphyxia (p. 509). They are not due to the interruption of inhibitory afferent impulses in the phrenics or vagi or sympathetics when the directly activated phrenic is cut or blocked (pp. 509, 510).

It is concluded that the crossed phenomenon reveals properties of neurone paths differing in quality from the conduction of nerve impulses: the opening of a new, hitherto unused path, and the tendency for such a path to remain open for relatively long periods of time (p. 510).

It is a pleasure to acknowledge our indebtedness to Dr. Hallowell Davis for suggesting the controls with curare and recording the action potentials of the phrenic nerves. To Dr. Henry G. Schwartz' surgical ability we owe the sections of dorsal roots in the rabbits.

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FUNCTIONAL BEHAVIOR OF COELIAC GANGLION CELLS OF THE RABBIT

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Of all indicators of nerve cell function the Nissl substance is undoubtedly the most easily observable. A long list of workers from the time of Hodge (1) to the present have reported a definite relationship between the size, arrangement and staining reactions of the Nissl substance and the functional behavior of the nerve cell in the central and autonomic nervous systems (2, 3, 4). Others have demonstrated that this material has a definite identity in the living cell and is segregated in the form of definite groups which constitute the Nissl granules (5, 6). In an effort to obtain further information concerning the behavior of the chromidial substance under normal and experimental conditions the coeliac ganglion cells of a series of pure-bred rabbits were studied. Twenty animals were employed in an examination of normal cells while 15 others were subjected to subcutaneous injections of nicotine. Procedures in the removal of the ganglia, fixing, staining and the method of counting the cells were the same as those used in the study of the albino rat (7).

OBSERVATIONS. *Types of cells.* It is possible, using a classification based upon the distribution and staining reactions of the Nissl substance, to separate the ganglion cells, as in the albino rat (7), into at least nine types. However, in order to facilitate analysis, these types were grouped into three classes which are similar to the three classes described for the albino rat. In the first class of cells the Nissl granules are large, evenly distributed throughout the cytoplasm and take a deep blue stain. The cells of class 2 always have a band of Nissl substance at the periphery of the cytoplasm. Cells with a perinuclear distribution of chromidial material, which were common in the rat in this class, rarely occur in the rabbit. Class 3 cells, like those of class 1, have a diffuse arrangement of the Nissl substance, but the granules are smaller, fewer in number and in consequence the cells stain less deeply. In addition some of the cells exhibit an almost complete lack of chromidial substance which is generally indicative of cellular degeneration or exhaustion.

Eccentric nuclei are a regularly recurring feature in the ganglion cells of the rabbit. In the normal Chinchillas 20 per cent of the cells in class 1

and 3, with 40 per cent in class 2, had peripherally placed nuclei. The other two breeds had even higher percentages of this type of cell.

Multinucleate cells are also common in the autonomic ganglia of the rabbit as in other rodents (8). They comprise from 20 to 30 per cent of all the cells and are found in all 3 classes. Such cells have been reported in man, especially in young persons, by Spiegel and Adolf (9). They state that these cells diminish in number with advancing age and are of the opinion that such cells are able to divide without nuclear changes. They believe that these cells are a part of the reserve system of the sympathetic nervous system. However, in the albino rat only a very few of such cells were seen.

TABLE 1

Cell counts in 3 breeds of normal rabbits

GROUP	BREED	TOTAL NUMBER ANIMALS	PERCENTAGE OF CELLS IN CLASS			TOTAL CELL COUNT
			1	2	3	
1	Havana	7	49.9	32.7	17.2	5428
2	Belted Dutch	5	48.6	36.9	14.3	4003
3	Chinchilla	8	37.7	45.8	16.3	6312

TABLE 2

Cell counts of Chinchilla rabbits injected with nicotine

GROUP	TOTAL NUMBER ANIMALS	DOSAGE IN MGM. PER KGM. BODY WEIGHT	INJECTIONS	PERCENTAGE OF CELLS IN CLASS			TOTAL CELL COUNT
				1	2	3	
4	3	Lethal	1 to 2	40.5	41.3	18.2	3106
5	5	1 mgm. every 30″	5 to 26	37.2	39.5	22.9	4982
6	1	2 mgm. every 30″	5	22.6	50.6	26.8	992
7	1	1 mgm. twice daily	42	35.3	46.6	17.6	1088
8	2	5 mgm. twice daily	30	30.4	50.9	18.7	2038
9	3	1 mgm. twice daily	112	25.1	58.0	16.7	3236

Differential cell counts. A summary of the percentages of cells that occur in each class in the normal rabbit is given in table 1. The number in each class in the Havana rabbit compares favorably with that in the albino rat. In the Chinchilla, and less so in the Belted Dutch, there are fewer class 1 and a greater number of class 2 cells than in the Havana. The percentage of class 3 cells in the three breeds of rabbits and in the albino rat is almost the same.

Langley (10) was one of the first to study the effects of nicotine on the autonomic nervous system but as yet little is known concerning its effect upon the nerve cells. With this fact in mind a group of rabbits were given subcutaneous injections of nicotine (table 2). The distribution of the

classes of cells in those animals receiving nicotine for a few hours or less is, with one exception, similar to that in the normal Chinchilla rabbit. Also the Nissl granules in the ganglion cells of this series were as large and distinct and stained as well as in the normal material. There was but little increase in the number of cells with eccentric nuclei. These observations seem to indicate that nicotine given over a short period of time has little effect on the functional activity of the nerve cell as evidenced by the appearance of the Nissl substance or the position of the nucleus.

Six animals (groups 7, 8 and 9) received injections of nicotine twice daily for two weeks or longer. In these groups there is a gradual decrease in the number of class 1 cells and an increase of class 2 cells in comparison with the normal Chinchilla rabbits. This constitutes a distinct shift from the diffuse, deep staining variety of cells to those with a peripheral band of Nissl substance. The chromidial substance in all three classes of cells often presented a picture of chromatolysis of a moderate or severe degree not unlike that described by Ma (11) for morphinized rats. In addition there is an appreciable increase in the number of cells having eccentric nuclei in all three classes of cells. These facts definitely indicate that nicotine administered for long periods of time produces changes in coeliac ganglia cells, which according to Dolley (2) and others, are evidences of functional activity.

SUMMARY

Based upon the characteristics of the Nissl substance at least three classes of cells occur in the coeliac ganglia of the rabbit. A quantitative analysis was made of these classes in 35 animals, comprising 3 breeds, with a total of over 30,000 cells.

Subcutaneous injections of nicotine given for short periods of time produced, in the Chinchillas, little change from the controls. However, when administered over a period of several weeks there was a shift from cells having an evenly distributed, deep staining Nissl pattern to cells with a peripheral band of Nissl substance. In addition moderate chromatolysis was of frequent occurrence together with an increase in the number of cells having eccentric nuclei. These facts indicate functional activity of a moderate degree.

From 20 to 30 per cent of the cells were multinucleated and a high percentage of cells having eccentrically placed nuclei were encountered in all the types of cells.

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THYROTROPIC EFFECT OF PITUITARIES FROM CRETIN RATS[1]

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In a previous paper (8), it was shown that following thyroidectomy in young rats, there occurred conspicuous histological changes in the pituitary. In a few weeks' time there accumulate large amounts of dense hyaline material within basophilic cells. These "thyroidectomy cells" are not "castration cells," because they occur in male cretin rats in which atrophy of gonads does not occur after thyroidectomy, and because when gonadectomy and thyroidectomy are performed at the same time in the same animal both "thyroidectomy cells" and "castration cells" occur in the pituitary and can be distinguished from each other by various morphological differences (10). A large number of rats have been studied since the publication of the above papers, and these cells have been found to occur constantly in all completely thyroidectomized rats and persist throughout the period studied, a year after operation. They also were seen in abundance in the pituitaries of thyroidectomized cats and dogs. Apparently the pituitaries of thyroidectomized rabbits do not show these cells, judging from the descriptions of Bryant (1) and of Marine, Rosen and Spark (6). The rat pituitaries also showed decrease in numbers of acidophiles, sometimes to a striking degree, sometimes only slightly. Factors which determine the fluctuations in the degree of acidophile loss have not yet been discovered.

With such obvious histological changes in the pituitary that are quantitative as well as qualitative, one would anticipate alterations in the production of the various pituitary hormones. A correlation of structural and functional changes might make it possible to ascribe the production of certain hormones to cells of specific type. Changes in various endocrines consequent to thyroidectomy might be due to the altered structure of the pituitary, rather than to any direct effect of the thyroid on other endocrines without the mediation of the pituitary.

It has been shown by a number of investigators that when rats are castrated the pituitaries which contain castration cells secrete into the blood stream an excess of gonadotropic hormone and store within the pituitary

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an excess of this hormone. The hyaline material within the castration cells might be looked upon as gonadotropic hormone which is stored when there is no end organ for it to act upon. Similarly the cells in the pituitary which react to thyroidectomy might be the cells which are producing the thyrotropic hormone, and the hyaline material might represent excess storage of the hormone.

EXPERIMENTAL PROCEDURE. Young immature rats were thyroidectomized, and an equal number of rats of the same sex and age and, in nearly all cases, of the same litter were kept as controls. Adequate thyroidectomy was indicated by 1, retardation of growth; 2, clear-cut changes in ratios of adrenal and gonad weights to kidney weights (9), and 3, increased weight of the pituitary in relation to body weight (8). When the desired time had elapsed after thyroidectomy, varying from 29 to 174 days, each pituitary was removed under aseptic conditions, weighed, suspended in salt solution, and drawn up into a sterile tuberculin syringe through a large bore needle, and injected into the leg muscle of a guinea pig. The guinea pigs were young females from 150 to 200 grams in weight. Injections of pituitaries from thyroidectomized rats were made on 3 or 4 successive days into different legs of the same guinea pig. A second guinea pig received pituitaries from the normal control rats of the same sex as the cretins over the same number of days, and two other guinea pigs were kept under the same environmental conditions in the same cage but received no pituitary injections. All the guinea pigs were killed on the day following the last injection, their thyroids weighed and microscopic sections made. A series of similar experiments was carried out, using in all 16 male cretin rats, 16 male controls, 13 female cretin rats, 13 female controls, 16 injected guinea pigs and 12 control uninjected guinea pigs. One experiment of the series is represented in table 1, which illustrates the method and expresses the comparison of male cretins with litter-mate female cretins, the comparison of cretin with control of the same sex, and also permits comparison of normal males with normal litter-mate females.

Rowland and Parkes (7) have studied carefully the various factors which must be considered in estimating the thyrotropic effect quantitatively. They have recorded data on the response of the guinea-pig thyroid to varying amounts of pituitary substance, the relation of thyroid weight to body weight of the guinea pig and the effect of the time intervals. According to their data the 4 and 5 day period which we chose is a desirable point on the ascending curve, and is long before refractoriness to the injections develops. Any fluctuations of the thyroid due to season, diet and environment was obviated by the fact that in each series of experiments there were two normal uninjected guinea pigs in the same cage, and such controls were taken as the standard of comparison for the injected.

RESULTS. Microscopically an intense hyperplasia was seen resulting from normal pituitary injections. This hyperplasia was manifested by almost complete disappearance of colloid; the cells changed in shape from

TABLE 1

DONOR RATS							RECIPIENT GUINEA PIGS						
Rat number	Sex	Age	Days after thyroidectomy	Body weight	Pituitary weight	Day of injection	Guinea pig number	Sex	Body weight	Thyroid weight	Thyroid/body weight of injected guinea pig = A / Thyroid/body weight of control guinea pig (average)	$\frac{A}{\text{Sum of body weights of rats}} \times 1000 = \text{Index}$	Microscopic hyperplasia
		days		*gms.*	*mgm.*				*gms.*	*mgm.*			
6Q6*	F.	203	165	147	17.9	1st	43	F.	154				
6Z8	F.	205	166	124	12.1	2nd							
7D5	F.	219	168	140	12.0	3rd							
7E8	F.	214	163	139	16.2	4th			149	44.0	1.47	2.7	++
				550	58.2								
6Q5	F.	203	Intact	172	10.9	1st	44	F.	149				
6Z7	F.	205	Intact	187	10.7	2nd							
7D6	F.	219	Intact	186	9.5	3rd							
7E4	F.	214	Intact	200	9.8	4th			148	34.5	1.21	1.6	++
				745	40.9								
6Q2	M.	203	165	161	9.4	1st	45	F.	167				
6Z2	M.	205	166	160	10.1	2nd							
7D4	M.	219	147	210	11.2	3rd							
7E1	M.	214	163	158	11.5	4th			164	49.5	1.52	2.2	++
				689	42.2								
6Q4	M.	203	Intact	245	7.0	1st	46	F.	167				
6Z4	M.	205	Intact	261	7.7	2nd							
7D2	M.	219	Intact	256	6.1	3rd							
7E3	M.	214	Intact	311	9.1	4th			169	53.5	1.68	1.6	++
				1073	29.9								
							47	F.	141				
									161	24.1			0
							48	F.	192				
									213	24.9			0

* The same alphabetic enumeration of rats indicates litter-mates.

flat to large cuboidal cells; there was an increase in number of cells as indicated by the very cellular appearance, their stratification, and some invagination into the lumen. This was accompanied by intense vas-

cularity seen grossly and microscopically. All of these conditions indicated that the experimental conditions were satisfactory for the demonstration of thyrotropic hormone.

The thyroids of guinea pigs receiving cretin pituitaries showed about the same intense hyperplasia histologically. Sometimes the cretin-injected guinea pigs seemed to show a little more hyperplasia; usually they showed a little less. The uninjected guinea pigs had normal thyroids with abundant dense colloid and flat cells and inconspicuous capillaries. The weights of the thyroids were regarded as a better index of stimulation than the histological appearance. The thyroids of all cretin-injected guinea pigs were increased in weight above those of the uninjected controls. Consequently the cretin pituitary contains abundant hormone. Usually,

TABLE 2

$$\frac{\text{Thyroid/body weight of injected guinea pigs}}{\text{Thyroid/body weight of uninjected guinea pigs (average)}} \text{ divided by sum of body weights of rats} = \text{Index of amount of hormone available per gram body weight of rat}$$

GROUP	PITUITARIES INJECTED OF CRETIN RATS	DAYS AFTER THYROIDECTOMY	INDEX	PITUITARIES INJECTED OF NORMAL CONTROL RATS	INDEX
1	1 male, 2 females	118, 46, 29	7.0	1 male, 2 females	5.6
2	3 females	84, 85, 86	6.6	3 females	3.9
3	4 females	165, 166, 168, 163	2.7	4 females	1.6
4	4 females	93, 79, 174, 92	4.0	4 females	1.9
5	3 males	56, 31, 52	5.2	3 males	5.1
6	4 males	91, 44, 99, 108	4.2	4 males	2.8
7	4 males	165, 166, 147, 163	2.2	4 males	1.6
8	4 males	131, 79, 169, 93	3.0	4 males	2.2
	16 rats		(Av.) 4.3	16 rats	(Av.) 3.1

however, the absolute weights of the thyroids of guinea pigs injected with cretin pituitaries were not as great as after normal pituitary injections.

If one considers that the dwarfed cretin rat has available for its small body almost as much hormone as does the larger rat of the same age, then the cretin has available more, not less, hormone for each gram of its body weight. This is expressed mathematically in the next to last column of table 1, where the factor of thyroid increase (A) is divided by the sum of the body weights of the rats whose pituitaries were used, in order to express quantitatively an index of the amount of hormone available for each gram of rat's body weight. It is a matter of judgment whether this calculation is justifiable. If one tentatively does consider the results in this light, then it will be seen in table 2 that this index is greater for the cretins than for the controls in every experiment without exception. The only case (group 5) in which the increase is negligible was an experiment in which the shortest time had elapsed after thyroidectomy. That this is a justifiable

calculation is indicated by the data in table 1 for the normal male and female rats. It will be seen that the normal males totalling 1073 grams caused an absolute weight of thyroid of 53.5 mgm., while the much smaller female rats of the same litters totalling 745 grams, gave a thyroid weight of only 34.5 mgm. Yet presumably with equal basal metabolic rates, there should be equal thyrotropic hormone in the two sexes. If now calculations are made for body weights, it will be seen that the index of both is 1.6, which is reasonable. Since such a calculation is necessary to give reasonable values in normals, it is logical to employ it in comparing dwarfed cretins with controls of normal size.

In table 2 the index is less when 4 pituitaries were injected than when 3 were injected, because the factor of thyroid increase was divided by a larger figure for body weights in the case of 4 rats. The female cretins showed a little more hormone than did the cretin males of the same litter and approximately equal post-operative period (groups 3 and 4 compared with 7 and 8).

Discussion. The cretin pituitaries are increased in size relative to body weight and usually by absolute weight due to the accumulation of hyaline material. Therefore the actual weight of pituitary substance injected was usually greater in the cretins than in the controls. The object of the experiment was to compare the cretin rat with its control irrespective of the weights of the pituitaries. However when calculations were made, each milligram of cretin pituitary contained about as much hormone as each milligram of control pituitary, expressed as index of hormone available per gram body weight of rat. In other words, increased amount of hormone available in the cretin is attained by increase in weight of pituitary.

Since no cell except the basophilic thyroidectomy cell is increased in cretin pituitaries, presumably this is the cell which produces any excess thyrotropic hormone.

The thyroidectomy cells appear histologically like hypersecreting cells, and if it proves correct to use the index discussed, the hypersecretion of thyrotropic hormone by the cretin pituitary is consistent with the histological changes. However, the index of increase does not seem as great as the histologically demonstrable amount of hyaline material would indicate. Perhaps this material is high in protein and contains relatively little hormone for its volume, just as "colloid goiter yields large amount of iodine-deficient thyroglobulin" (3).

It is reasonable to expect hypersecretion of thyrotropic hormone in a hypothyroid animal. When there is a lack of thyroid secretion it is to be expected that there should be a compensatory hyperactivity of the pituitary cells which form thyroid stimulating hormone, which would act upon any remnant of thyroid tissue that was present, this being in accordance with what we see in many compensatory reactions which tend to maintain

equilibrium. The thyroid-pituitary relationship must be a self-limiting process under usual conditions. It has been shown that feeding thyroid extract to rats reduces the thyrotropic effects of their pituitaries to a minimum (4). A reasonable conception would seem to be that when the pituitary stimulates the thyroid to secrete, its thyroxin in turn acts upon the pituitary suppressing its secretion of thyrotropic hormone until a fall in thyroxin results in the pituitary resuming its secretion. In the cretin with no thyroxin there is no inhibition of the pituitary and it continues to secrete unchecked.

Several previous studies of thyrotropic effect of pituitaries from thyroidectomized animals have been made. Hohlweg and Junkmann (4) studied the effect of pituitaries of rats thyroidectomized 5 to 20 days previously. They had 25 to 50 per cent mortality after thyroidectomy. This must have been due to some complicating factor such as parathyroid removal or trauma of recurrent laryngeal nerves; for when we avoided these, no fatalities occurred. In our experience 20 days is too short a time after operation to permit more than the beginning histological changes in the pituitary. They give no weights of injected guinea-pigs' thyroids. Judging from the histological appearance of the thyroid after varying doses of pituitary substance were given, they concluded that there was no increase in thyrotropic effect of thyroidectomized rats' pituitaries.

Houssay, Novelli and Sammartino (5) injected into guinea pigs the pituitaries of adult rats of 200 grams, one month and a half after thyroidectomy. The average weights of the guinea-pig thyroids were 30 mgm. for the uninjected controls, 43 mgm. for those injected with normal rat pituitaries, and 34 mgm. for those injected with pituitaries from thyroidectomized rats, and they concluded that there was no increase in the thyroid stimulating effect after thyroid ablation. They gave two injections of one and a half lobes each and killed the guinea pigs on the 3rd day.

Ch'en and Van Dyke (2) studied rabbits. Since there are so many species differences in the reaction of the pituitary, data on rabbit pituitaries are perhaps irrelevant to the present experiments on rats. They found in 45 ± 5 per cent of paired guinea pigs, the pituitaries of male thyroidectomized rabbits caused greater stimulation of the thyroid than normal pituitaries and in 72 ± 17 per cent of guinea pigs the pituitaries of female thyroidectomized rabbits caused greater stimulation than did normal pituitaries. They felt additional experiments were necessary before conclusions could be definite.

SUMMARY

The thyrotropic hormone content of rats' pituitaries was tested by injecting whole pituitaries on successive days into guinea pigs and noting the effect upon the weight and histological picture of the thyroids.

When thyroidectomy carried out on young rats was adequate (as in-

dicated by dwarfing, increase in weight of the pituitary in relation to body weight, and alterations in ratio of the weights of the adrenals and gonads to the weights of kidneys), the pituitaries contained abundant thyrotropic hormone; but their injection usually caused less increase in absolute weight of the guinea-pig thyroid than did the injection of pituitaries of normal age controls.

If one considers the amount of hormone available to the rat per gram of its body weight, and calculates an index to express this, the index is greater for the cretins than for the controls in every experiment.

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THE ORIGIN OF FECAL FAT IN THE ABSENCE OF BILE, STUDIED WITH DEUTERIUM AS AN INDICATOR[1]

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When bile is prevented from flowing into the intestinal tract either by a bile duct obstruction or by a bile fistula, the amount of fat in the stools is invariably increased, even though considerable amounts of fat are absorbed (1, 2).

It has also been shown, in starvation experiments on humans, that fat may be secreted into the intestinal tract (3). The important rôle of fat secretion into the intestinal lumen was further experimentally demonstrated on complete bile-fistula dogs kept on a fat-free diet (4). These dogs excreted even more fat than did the control animals, the bile of which ran into the intestinal tract.

This suggests the following question. In humans with complete bile fistula, what fraction of the increased fecal fat represents unabsorbed food fat and how much represents secreted fatty acids?

The new method of labelling physiological compounds such as fatty acids by the introduction of the heavy isotope of hydrogen (deuterium) into the molecule (5), offered the possibility of attacking this problem directly in humans.

Organic molecules in which a part of the hydrogen is replaced by this isotope are, chemically and physiologically, almost identical with their natural analogues. The animal cell is not able to distinguish one from the other. A number of physiological questions have already been solved by the use of such deuterium-containing organic molecules.

If patients with a bile fistula are given a deuterium-containing fat together with the diet, the deuterium content of the fecal fatty acids should indicate how much of this fat has been absorbed, and it can be calculated whether the increase of fecal fatty acids in such patients is derived from the food fatty acids or from secretions.

Two otherwise healthy patients with bile fistulae previously reported

[1] Part of this work was carried out with the support of the Josiah Macy, Jr., Foundation.

upon (6, 7) offered a unique possibility for such experiments. When the bile drainage tube of these patients was opened, no trace of bile flowed into the intestine, as indicated by the complete absence of bile pigments from the stools. On the other hand, when the drainage tube was closed, all bile flowed into the intestine. By alternately opening and closing the drainage tube it was possible to obtain periods with complete absence or presence of bile in the intestine.

The patients were kept on a standard diet, the fat content of which was previously determined. Each experimental period was marked at the beginning and end by giving 0.5 gram of carmine by mouth. Several days were allowed to elapse between periods.

The fat given to the patients contained 4.37 atom per cent deuterium. It was prepared from linseed oil according to Schoenheimer and Ritten-

TABLE 1

Stool fatty acids in bile-fistula humans

(The fat (β71) contained 4.37 atom per cent deuterium)

PATIENT	PERIOD NUMBER	DURATION OF PERIOD	BILE FLOW	FAT β71 ADDED PER DAY	TOTAL FATTY ACIDS IN DIET PER DAY	FATTY ACIDS IN STOOLS PER DAY	AMOUNT OF FAT β71 RECOVERED FROM STOOLS
		days		*grams*	*grams*	*grams*	*per cent*
D. W.	1	10	Into intestine		8.4	1.23	
D. W.	2	10			8.1	1.99	
D. W.	3	6	Outside	0.75	8.5	8.2	35
D. W.	4	10	Outside		8.1	9.43	
H	5	5	Outside	2.1	6.0	6.22	30
H	6	5	Into intestine		4.0	0.73	

berg (8) and had properties similar to those of olive oil. It was given to the patients in divided doses on bread with their meals on the middle days of the intervals studied.

METHOD OF ANALYSIS. The combined stools for each interval were acidified with glacial acetic acid and dried by powdering with anhydrous sodium sulphate. About 500 gram aliquots were continuously extracted for 24 hours with ether in a modified Clarke extractor. The ether extract was then washed free of acetic acid and sulphate in a separatory funnel with water and dried to constant weight on a steam bath. The dried ether extract was then saponified by refluxing for two hours on a steam bath with 7 per cent potassium hydroxide in 90 per cent methyl alcohol and transferred to a separatory funnel by washing with successive portions of water and ether. More ether and water were added and the unsaponifiable

fraction removed by repeated extraction with ether. The combined ether extract was washed once with water and this water, combined with the original aqueous layer, was strongly acidified with concentrated HCl. The fatty acids were extracted with successive portions of ether, and the combined ether layers were washed with water until neutral to litmus and dried to constant weight on the steam bath.

The deuterium analysis of the fatty acids was done according to Rittenberg and Schoenheimer (9). The results are given in table 1. Melting points and iodine numbers of the fatty acids were determined but, since the results seem to have no bearing on the problem, they have been omitted.

Discussion. Our findings confirm the results of previous workers who showed that when bile is absent from the intestinal tract, the amount of fatty acids in the stools increases. In the periods when bile flowed into the intestinal lumen, the amount of fatty acid in the stools was only a small fraction of that in the diet (15–25 per cent). When the bile ran to the outside, the amount increased to about the same as was given with the diet.

From these findings it might be concluded that no fatty acid was absorbed, and that in these periods the fecal fatty acid represented in its entirety the unabsorbed food fatty acid. That this is not the case is clearly indicated in the experimental periods when the food fatty acid was labelled by the addition of small amounts of deuterium-containing fat (DW3 and H5). While in these periods also the total amounts of fecal fatty acid and food fatty acid were approximately the same, the greater part of the fecal fat must have had another origin. (It contained only 35 and 30 per cent of the deuterium administered with the diet fat.) It can be concluded from these analyses that the remaining 65 to 70 per cent of the diet fatty acid was absorbed and that the greater part of the fecal fat in our patients originated from the fat secreted into the intestinal tract.

SUMMARY

The amount of fecal fatty acid has been compared with the amount of diet fatty acid in two patients with bile fistulae. In the periods when bile did not enter the intestinal tract, the amount of fecal fat was greatly increased. By adding small amounts of deuterium-containing fats to the diet, it was shown that 65 to 70 per cent of the diet fatty acids was absorbed in the absence of bile and that the increase of fecal fat was due to fats secreted into the intestinal lumen.

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A COMPARISON OF THE CHEMICAL COMPOSITION OF STIMULATED AND RESTING SALIVA OF CARIES-FREE AND CARIES-SUSCEPTIBLE CHILDREN[1]

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Few studies have been made on the chemical composition of resting saliva. On the other hand, many reports have appeared concerned with the composition of stimulated saliva (paraffin chewing). Little is known as to whether there is any relationship existing between the method of collection of the saliva and the amounts of substances present (1). The variable results of analyses have been discussed by Becks and Wainwright (2), who have emphasized the possibility of explaining these discrepancies by variations in analytical procedure or method of collection of the saliva. Hubbell and Bunting (3) and Hubbell (4), who have reported on the calcium and phosphorus content of stimulated salivas of a large number of children, have been criticized by Becks and Wainwright on the basis that stimulated salivas were used. Wainwright (5) later reported a new technique for the collection of the saliva as well as improved modifications in the determination of calcium. In view of the criticism of Becks and Wainwright and the paucity of comparative data on resting and stimulated saliva, a study was undertaken to obtain such data.

Since we were interested for some time in the possible relationship between the chemical composition of saliva and dental caries, we have studied children with a history of pronounced dental caries and those apparently immune. The subjects used were public school children of known dental history ranging in age from 9 to 16 years. Most of them had been under observation for several years. The children were brought to the laboratory by automobile at 6:30 in the morning. They were previously instructed not to brush their teeth or to partake of foods or liquids. They were allowed to rest 15 minutes more before the actual

[1] A preliminary report of this investigation was presented before the International Association for Dental Research at the 14th Annual Meeting in Louisville, March, 1936.

[2] Study made as a part of the Dental Caries Research supported by the Rackham Foundation in the School of Dentistry, University of Michigan.

collection of the saliva was started. The resting saliva was collected by having the child tip his head forward with mouth open and allowing the

TABLE 1

The analysis of stimulated and unstimulated saliva of children

Each figure represents an average of the value obtained on analysis of 4 different samples of saliva. The analytical values unless otherwise indicated are expressed as milligrams or cubic centimeters per 100 cc. of saliva. R = resting; S = stimulated.

SUBJECT	AGE	VOLUME		SOLIDS		ASH		CALCIUM		PHOSPHORUS		CARBON-DIOXIDE		pH	
		R	S	R	S	R	S	R	S	R	S	R	S	R	S
Caries-free															
	yrs.	*cc./min.*	*cc./min.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*cc.*	*cc.*		
1	13	1.05	2.7	243	366	85	133	6.38	5.83	15.3	13.6	12.9	13.6		
2	13	0.32	1.9	415	633	100	130	5.00	4.50	18.1	14.0	17.1	64.0		
3	14	0.59	1.8	324	498	91	115	5.77	4.38	16.5	11.6	24.3	60.9		
4	11	0.39	0.96	312	397	98	158	6.00	5.36	16.5	15.6	32.5	61.1		
5	12	0.49	2.3	377	491	101	115	6.32	5.09	17.5	13.3	11.7	54.2		
6	16	0.28	1.1	520	416	83	96	5.63	3.91	14.2	12.5	7.4	23.2	6.6	7.1
7	9	0.32	0.96	533	560	127	105	6.21	4.63	16.8	12.2	6.4	33.5	6.7	7.5
8	9	0.73	1.3	363	450	101	88	7.30	5.60	15.5	12.4	10.2	29.2	6.7	7.3
9	13	0.63	0.66	427	645	100	120	6.80	5.91	15.1	13.7	5.9	41.0	6.6	7.0
10	15	0.45	2.4	408	571	157	122	7.12	5.43	16.5	14.6	4.4	29.0	6.6	7.1
11	12	0.31	0.59	401	342	113	83	5.40	3.93	15.4	12.8	9.5	23.0	6.5	7.3
12	13	0.19	1.8	560	360	137	92	6.71	5.14	14.1	12.3	20.0	29.8	6.6	6.9
Av.	12.4	0.46	1.52	407	472	108	113	5.79	4.99	16.0	13.3	10.3	40.6	6.6	7.17
Caries-susceptible															
13	11	0.40	1.2	357	451	108	132	4.94	4.57	14.2	10.9	9.9	40.2	6.7	7.3
14	14	0.23	0.63	430	357	91	68	5.30	3.91	16.1	12.9	6.3	24.3	6.8	7.1
15	14	0.35	1.5	335	445	125	135	5.35	5.00	17.1	13.8	5.9	31.8	6.6	7.1
16	13	0.47	1.4	278	293	107	107	5.01	4.68	16.6	13.3	12.0	27.2	6.8	7.2
17	13	0.51	0.96	278	293	92	83	6.02	4.56	14.6	12.8	13.3	31.0	6.6	7.1
18	14	0.41	1.7	333	440	95	110	6.10	5.23	17.2	13.4	9.5	43.3	6.7	7.3
19	12	0.40	1.5	352	542	90	100	6.32	4.99	14.2	11.3	13.5	42.0	6.7	7.4
20	13	0.38	1.5	348	482	53	83	5.17	4.37	17.7	12.9	10.3	43.1	6.5	7.3
21	10	0.35	2.5	425	455	87	90	5.98	4.33	18.4	14.5	5.6	25.2	6.8	7.3
22	13	0.67	1.6	580	522	97	105	6.84	5.70	14.6	10.8	5.6	35.0	6.8	7.4
23	13	0.27	1.5	460	435	117	105	6.49	5.12	16.0	12.3	6.6	40.9	6.7	7.4
24	10	0.45	1.3	298	350	90	86	6.84	5.70	15.6	12.9	21.1	49.2	6.8	7.2
25	9	0.40	1.5	360	370	105	90	6.24	5.01	14.7	13.4	7.7	29.3	6.4	6.8
Av.	11.5	0.40	1.45	373	419	96	99	5.89	4.87	15.9	12.9	9.8	35.6	6.6	7.26

saliva to flow from his mouth without the movement of tongue or jaws. After some practice the subject became adjusted to this position and very

little trouble was encountered in collecting the sample. The saliva was allowed to drip into a small glass funnel resting in a graduated cylinder which contained a layer of paraffin oil. True resting saliva was always water clear and almost totally devoid of cell débris. The movement of the tongue or jaws or yawning caused the saliva to become cloudy. When such was the case, the sample was discarded. The time elapsed and the amount of saliva collected were carefully noted. After 15 to 18 cc. were collected, the subject was allowed to rest 15 minutes, and then a second 15 to 18 cc. sample was collected by paraffin stimulation. On several days the process was reversed, i.e., the stimulated sample was collected first. This was done in order to determine whether or not there was a fatigue factor. Collection of the resting and stimulated salivas on two consecutive days was also tried. Since no significant differences related to the order of collection were found, the procedure finally adopted was the collection of both samples the same morning with a 15 minute rest interval between each collection.

Four 1 cc. samples were removed immediately after collection and the pH (colorimetric) and the carbon dioxide capacity (Van Slyke and Cullen (6)) were determined. The remainder of the saliva collected was centrifuged under oil at a high speed for 30 minutes. Two 2 cc. samples were removed and transferred to a platinum dish and dried at 110° overnight in an electric furnace to determine total solids. The dried material was then ashed in an electric muffle at a temperature between 450 and 500°. After weighing, the ash was dissolved in 1 cc. of HCl (1:3) and the solution was transferred to a 25 cc. volumetric flask and made up to volume. Aliquots of this were used for phosphorus determinations by the method of Fiske and Subbarow (7). Two 6 cc. samples of the centrifuged saliva were transferred to a test tube, treated with 6 cc. of 20 per cent trichloroacetic acid, shaken and allowed to stand 10 minutes. The precipitate formed was removed by filtration and the filtrate analyzed for calcium by the procedure outlined by Wainwright (5) using the Halverson-Bergeim (8) procedure.

Four separate samples were obtained from each individual. There was very little variation between individual samples so that the data in the table represent an average of 4 determinations. Since 25 subjects were used the analyses include a total of 100 samples. It is true that there is a higher concentration of calcium and phosphorus in the resting saliva than in the stimulated but examination of the total solids reveals that with few exceptions (3 in each group) the solids are lower in the resting salivas. The carbon dioxide capacity was lower in the resting than in the stimulated salivas. The values obtained for the stimulated salivas are in accord with those reported by Hubbell and are in the same range as those of Krasnow (9).

Our results do not indicate that the composition of either resting or stimulated saliva is altered by the factor of caries susceptibility.

The cases for this study were selected with the help of Dr. Philip Jay.

SUMMARY

Analyses have been made on the resting and stimulated salivas of 25 children, of whom 12 were caries-free and 13 caries-susceptible.

The earlier work of Hubbell and Bunting on stimulated saliva has been verified.

Resting salivas contained a higher calcium and phosphorus content and a lower carbon dioxide capacity than activated salivas.

There is no apparent relationship between chemical constituents of the salivas studied and dental caries.

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GASTRIC ACIDITY FOLLOWING PARTIAL GASTRECTOMY AND VAGOTOMY

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In a previous publication (1) it was shown that whole stomach pouches from which the pylorus had been removed secreted, on an average, only about $\frac{1}{5}$ to $\frac{1}{6}$ as much acid as whole stomach pouches in which the pylorus was intact. It was also shown that removal of the pylorus and anastomosis of the fundus to the duodenum (Polya type) caused a lowering of acidity which was definitely greater than could be accounted for by the diluting and neutralizing effects of the duodenal secretions entering the stomach. When atropine (0.05 mgm. per kilo) was given intramuscularly to these animals total anacidity resulted in each of six experiments. As a result of these studies it was concluded that the pyloric portion of the stomach is a factor of paramount importance in the intragastric chemical phase of acid secretion and that a large part of the acid secretion which may persist after pyloric removal is cephalic (not psychic) in origin. In the present article we wish to present studies on dogs before and after partial gastrectomy and double vagotomy.

METHODS. A two per cent Liebig's extract test meal containing 15 mgm. of phenol red per liter was used in all experiments (2). The manner of performing the experiments and the management of the animals was the same as that previously described. Four dogs were studied. From 6 to 8 satisfactory fractional gastric analyses were first done on each animal. Both vagus nerves were then cut in the thorax. After recovery the animals were tested to demonstrate the absence of the psychic phase of acid secretion. The pyloric portion of the stomach was then removed and the fundus anastomosed to the duodenum according to the Polya method. A second series of fractional analyses was then performed. The present experiments cover a period up to 3 months after the second operation.

RESULTS. I. *The emptying time of the stomach.* In previous studies following partial gastrectomy without vagotomy (1), it was found, in agreement with the work of others, that the emptying time of the stomach was shortened, usually by about one-half hour. In the present studies

more rapid emptying did not occur, and on all four animals the duration of most of the experiments was the same as before operation (2 hrs.). This finding definitely eliminates a more rapid emptying time as a factor in the results.

II. *Gastric acidity.* In figure 1 the average acidity of the total fluid secretions entering the stomach (lower half) and the average acidity of the gastric contents (upper half) before and after operation are shown on

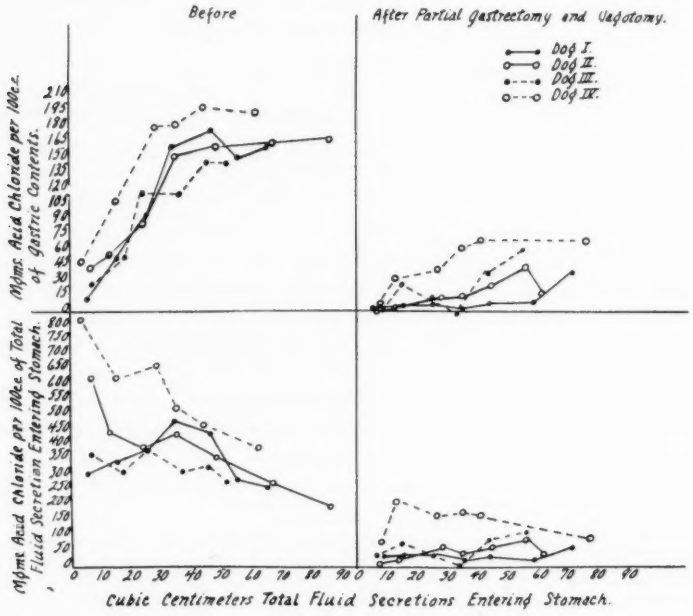
Before
After Partial Gastrectomy and Vagotomy.
Dog I.
Dog II.
Dog III.
Dog IV.
Mgms. Acid Chloride per 100 c.c. of Gastric Contents.
Mgms Acid Chloride per 100 c.c. of Total Fluid Secretion Entering Stomach.
Cubic Centimeters Total Fluid Secretions Entering Stomach.

Fig. 1. Shows the average acidity of the gastric contents and of the total fluid secretions entering the stomach on four dogs before and after partial gastrectomy and vagotomy. The samples were grouped according to increasing amounts of total fluid secretions entering the stomach and the average acidity for each group plotted. The analysis is based on 103 half-hour gastric samples obtained before operation and 145 half-hour samples obtained after operation.

each of the four dogs. The samples before and after operation were grouped according to increasing amounts of total fluid secretions entering the stomach, identical groupings being used for the experiments before and after operation, and the average acidity for each group plotted. The acidity of the gastric contents as well as the acidity of the total fluid secretions entering the stomach were profoundly lowered after operation.

In table 1 a few typical experiments are shown on dog II. In the first

four experiments tabulated after operation almost complete anacidity occurred. The acid deficits are due to the fact that since no acid was being secreted, the acidity of the test meal was lowered by neutralization by the non-acid secretions entering the stomach. Experiments in which anacidity occurred were common on all four dogs. The frequency was as follows:

Dog I, 8 out of 16 experiments or 50 per cent
Dog II, 4 out of 14 experiments or 29 per cent
Dog III, 5 out of 14 experiments or 36 per cent
Dog IV, 0 out of 7 experiments or 0 per cent

Hence in 51 experiments after operation 17 or 33 per cent were characterized by anacidity. Dog IV which had the highest preoperative acidity curve which we have ever encountered in a normal dog (due apparently to water absorption (2)) never showed complete anacidity. In the other three dogs anacidity was often encountered even when a second experiment was started immediately after terminating the first experiment (expts. 2 and 3, table 1). In the remaining experiments on all four dogs a low secretion of acid occurred as illustrated in experiments 5, 6, 7, 8 and 9 in table 1.

III. *The response to histamine and to the intestinal phase.* As shown in table 1 a marked secretion of acid occurred in response to histamine administered intramuscularly in dog II. Dog I gave a less marked response, while dog III gave no response. Dog IV was not studied.

During the course of the experiments the animals were purposely placed on a regimen of forced feeding (4 feedings daily) with as much raw ground liver as they would eat, the last feeding being given 24 hours before performing an experiment. After approximately 10 days on this regimen it was observed that the amount of acid secreted was definitely elevated above the previous low value in all four dogs. This effect was sometimes found to be present for as long as 48 hours after the last meat feeding. After discontinuing the forced feeding regimen and placing the animals on the usual single daily feeding of milk and dog biscuits, it required approximately 5 days for the acid secretion to return to its previous low level. This effect is due to either (or both) a general cellular stimulation resulting from the luxus consumption of protein or a greatly prolonged intestinal phase. The latter factor was definitely proven since in all dogs food remnants were found in the duodenal secretions which regurgitated into the stomach even as long as 48 hours after the last meat feeding. The more sluggish intestinal movements probably resulted in food remaining in the upper intestine longer than normal and this in conjunction with the low acidity of the gastric contents entering the intestine, resulted in an unusually prolonged intestinal phase.

TABLE 1

A series of experiments on dog II after partial gastrectomy and vagotomy compared with an average of 7 experiments before operation

P.S.P. PER CENT	MGM. EXTRA ACID CHLORIDE PER 100 CC. GASTRIC CONTENTS	MGM. EXTRA ACID CHLORIDE PER 100 CC. OF SECRETION	TOTAL FLUID	ACID FLUID	NON-ACID FLUID	PER CENT ACID FLUID	TIME	BILE	REMARKS	
89	36	327	11	6	5	55	½	0	Average of 7 fractional analyses	Before operation
80	82	410	20	14	6	70	1	+		
59	125	305	41	21	20	51	1½	++		
43	188	330	57	31	26	54	2	++		
93	−4	−57	7	0	7	0	½	+		
88	−11	−92	12	0	12	0	1	++++		
64	+4	+11	36	0.7	35.3	3	1½	++++		
64	−8	−22	36	0	36	0	2	++++		
88	−12	−100	12	0	12	0	½	+++	800 cc. test meal	
62	+10	+26	38	4	34	11	1	+++	followed by 800	
70	−16	−53	30	0	30	0	1½	++++	cc. test meal	
...	.....	.....	...	...	...	...	...	.....		
89	−8	−73	11	0	11	0	½	++++		
88	−14	−117	12	0	12	0	1	++++		
92	−34	−425	8	0	8	0	1½	+++		
94	−3	−50	6	0	6	0	½	++		
86	+5	+36	14	1	13	7	1	+++		
72	−4	−14	28	0	28	0	1½	++++		
64	−6	−17	36	0	36	0	2	++++		
79	+7	+33	21	1	20	5	½	+++		
66	+20	+59	34	3	31	9	1	+++		
56	+12	+27	44	2	42	5	1½	++++		
52	−2	−4	48	0	48	0	2	++++		
72	+17	+61	28	3	25	11	½	++		After partial gastrectomy and vagotomy
62	+31	+82	38	5	33	13	1	++		
62	+26	+68	38	4	34	11	1½	++		
48	+46	+89	52	8	44	15	2	++		
71	+10	+35	29	2	27	7	½	+++		
53	+30	+64	47	5	42	11	1	+++		
40	+40	+67	60	7	53	12	1½	++		

TABLE 1—*Concluded*

P S P PER CENT	MGM. EXTRA ACID CHLORIDE PER 100 CC. GASTRIC CONTENTS	MGM. EXTRA ACID CHLORIDE PER 100 CC. OF SECRETION	TOTAL FLUID	ACID FLUID	NON-ACID FLUID	PER CENT ACID FLUID	TIME	BILE	REMARKS
82	+2	+11	18	0.3	17.7	2	½	+++	900 cc. test meal
59	+23	+56	41	4	37	10	1	+++	followed by 700
38	+18	+29	62	3	59	5	1½	+++	cc. test meal
...	...	...	...	...	...	...	...	...	...
83	−2	−12	17	0	17	0	½	++++	
56	+46	+105	44	8	36	18	1	++++	
85	+14	+93	15	2	13	13	½	++	Intestinal phase
78	+72	+328	22	12	10	55	1	+++	
62	+64	+169	38	11	27	29	1½	+++	
54	+88	+191	46	15	31	33	2	++	
76	+19	+79	24	3	21	13	½	+++	1 mgm. histamine
56	+96	+218	44	16	28	36	1	+++	
38	+142	+229	62	24	38	39	1½	+++	1 mgm. histamine
20	+238	+298	80	40	40	50	2	++++	

The responses to histamine and to the intestinal phase show that the acid secreting glands were capable of responding to an adequate stimulus in a normal manner.

IV. *The rôle of the duodenal secretions in producing the lowered acidity.* The method of evaluating the part played by the duodenal secretions in lowering the acidity of the total secretions entering the stomach has been described in detail in a previous article (1) and will only be briefly outlined here. Average curves for the *total fluid*, *non-acid fluid* and *acid fluid* entering the stomach before and after operation were plotted on coördinate paper and the areas under the respective curves determined by means of a standardized planimeter and converted into terms of cubic centimeters of the various fluids. These values for the four dogs before and after operation are shown in table 2. It is seen that in dogs I and IV there was a slight increase in the amount of the total fluid secretions entering the stomach after operation which is considerably less than was previously found after partial gastrectomy without vagotomy (1). In dogs II and III the amount of total fluid entering the stomach after operation was slightly less than before operation but in spite of this the acid fluid was 44 per cent lower than before operation. This finding definitely eliminates an increase in the duodenal secretions entering the stomach as the

sole cause of the lowered acidity after operation in these two dogs and shows that considerably less acid fluid must have been secreted.

Three calculations were previously described (1) which were used to determine the exact rôle of the duodenal secretions in producing the lowered acidity. These calculations and their application to the present experiments are as follows:

Calculation I. In this the increase in total fluid entering the stomach after operation is assumed to be due to duodenal secretions. The neutralizing effect of the increased duodenal secretions is ignored but a calculation is made to determine how much lowering of acidity could be brought about by dilution alone. In dogs II and III this calculation is unnecessary, since the per cent of acid fluid is 44 per cent lower after operation with no increase in total fluid. In dogs I and IV the per cent of acid fluid after operation is 48 and 49 per cent lower than the diluting effect of the increased total fluid could account for. In previously reported studies after partial gastrectomy without vagotomy (1) the acidity was from 32 to 40 per cent lower than calculated.

TABLE 2

Shows the average composition of the total fluid secretions entering the stomach in four dogs before and after partial gastrectomy and vagotomy

The values were determined as explained in the text

DOG	BEFORE	AFTER
I	Total fluid = 105 cc. Non-acid fluid = 46 cc. Acid fluid = 59 cc. Per cent acid fluid = 56	Total fluid = 113 cc. Non-acid fluid = 108 cc. Acid fluid = 5 cc. Per cent acid fluid = 4
II	Total fluid = 99 cc. Non-acid fluid = 44 cc. Acid fluid = 55 cc. Per cent acid fluid = 56	Total fluid = 97 cc. Non-acid fluid = 85 cc. Acid fluid = 12 cc. Per cent acid fluid = 12
III	Total fluid = 74 cc. Non-acid fluid = 34 cc. Acid fluid = 40 cc. Per cent acid fluid = 54	Total fluid = 73 cc. Non-acid fluid = 66 cc. Acid fluid = 7 cc. Per cent acid fluid = 10
IV	Total fluid = 103 cc. Non-acid fluid = 21 cc. Acid fluid = 82 cc. Per cent acid fluid = 80	Total fluid = 108 cc. Non-acid fluid = 79 cc. Acid fluid = 29 cc. Per cent acid fluid = 27

Calculation II. In this the increase in total fluid entering the stomach after operation is considered to be due to duodenal secretions. In addition

to determining the diluting effect, as in calculation I, the neutralizing effect is also evaluated by assuming that the duodenal secretions have an average alkalinity of 0.04 normal and adding the amount of acid fluid that could have been neutralized to the amount observed. In dogs II and III the calculation is unnecessary. In dogs I and IV the per cent of acid fluid after operation is 46 and 48 per cent lower than could be accounted for by both the diluting and neutralizing effects of the increased duodenal secretions entering the stomach. After partial gastrectomy without vagotomy (1) the values ranged from −25 to −38 per cent.

Calculation III. In this allowance is made for the neutralizing effect of the *non-acid fluid* in the gastric samples both *before* and *after* operation by considering it to have an alkalinity of 0.04 normal and adding the acid that could have been neutralized to that observed. If the total fluid entering the stomach after operation is increased the diluting effect is also evaluated as in calculation I. When this calculation was applied to the present series of experiments on four dogs the acid fluid was found to be 36, 35, 37 and 38 per cent lower than could be accounted for. In previous experiments after partial gastrectomy without vagotomy the values ranged from −20 to −32 per cent.

IV. *The consistency of the gastric contents.* After operation the consistency of the gastric contents in dogs I, II and III underwent a profound change due to the presence of large amounts of mucus of about the consistency of raw egg albumin. This was more pronounced in the present series than in partial gastrectomy without vagotomy. The viscosity was often so great that considerably difficulty was experienced in pipetting the samples. There was a very evident correlation between the amount of mucus and the amount of acid secreted. In experiments with anacidity the amount of mucus was very great but when the acid secretion was high (in response to histamine or the intestinal phase) the mucus was often not detectable by gross examination or was greatly reduced. In vitro experiments showed that this mucus was soluble in N/10 hydrochloric acid. This change in the consistency of the gastric contents was not previously observed in whole stomach pouches from which the pylorus has been removed even when the acid secretion was very low (1).

Discussion. The acid secretion following partial gastrectomy and vagotomy is definitely less than was previously found after partial gastrectomy alone (1) thus confirming the statement that part of the acid secreted after partial gastrectomy may be due to the cephalic phase. Studies were not made after vagotomy alone but the work of other investigators has shown that the decrease while definite is not marked, hence most of the lowering observed in the present experiments was due to pyloric removal and consequent interference with the intragastric chemical phase of acid secretion. The cause of the slight secretion which often

occurred is not clear. Since the acid secreting glands were fully capable of responding to adequate stimuli it is quite possible that a summation of several minimal stimuli such as mechanical distention, presence of duodenal secretion in the stomach, etc., were capable of exciting a low rate of secretion. The intestinal phase may also have been active even when the usual single daily feeding of milk and dog biscuits was given.

The increased sensitivity of these animals to the intestinal phase is important and suggests that some of the confusion in the literature on partial gastrectomy may be due to the failure to eliminate the intestinal phase. Unpublished experiments (3) have shown that the presence of acid gastric contents in the intestine definitely inhibits the intestinal phase of acid secretion hence it is quite likely that the low acidity of the gastric contents after partial gastrectomy renders the intestinal phase more pronounced than in normal animals.

Priestly and Mann (4) have suggested that the more rapid emptying time of the stomach which usually occurs after partial gastrectomy is a factor in producing the lowered acidity. Since a more rapid emptying time did not occur in the present experiments, it can be dismissed as a factor of importance.

The average increase in the total fluid secretions entering the stomach after operation was very small in two animals (5 and 8 per cent) while no increase occurred in two others, yet in all four animals the average acidity of the gastric secretions and gastric contents was profoundly lowered. This finding in itself definitely eliminates an increase in duodenal secretions entering the stomach as the sole cause of the lowered acidity after partial gastrectomy. A more detailed analysis shows that the average acidity of the gastric secretions was from 35 to 49 per cent lower than could be accounted for by the diluting and neutralizing effects of the duodenal secretions.

The presence of the very viscid mucus noted in these and previous experiments has to our knowledge not been described before, possibly because of the frequent use of a meat test meal which would obscure it. When large amounts of acid were secreted in response to histamine or the intestinal phase the mucus was not observed possibly because it is soluble in acid. This fact suggests that the mucus is a *result* and not the *cause* of the low acidity. If this viscid mucus occurs in the human stomach after partial gastrectomy, it may be an important factor in hastening the healing and in preventing the recurrence of peptic ulcer.

The acid values given in the present paper are for *total acid*. The gastric samples were, on several occasions, tested for so called "free acid" with Topfer's reagent but were always negative. It is important to note that in the four animals studied the average *total acidity* of the gastric contents after operation was always definitely below the threshold

value of *free acid* necessary for the digestion of living tissues (97 to 146 mgm. of acid chloride per 100 cc.) as determined by Dragstedt (5). It is thus quite likely that experimental jejunal ulcer would not have occurred in these animals following the Mann-Williamson operation.

The gastric acidity curve in response to a Liebig's extract test meal in partially gastrectomized animals before and after double intrathoracic vagotomy, constitutes a new preparation which may prove useful in studying the magnitude of the cephalic (not psychic) influence on gastric acidity. The evidence indicates that it may vary considerably in different animals.

The duration of the lowered acidity was not specifically studied but it was shown to persist for at least three months with no evidence of a return to normal. This result is not in agreement with the findings of Shapiro and Berg (6) who noted a beginning return to normal in from 12 to 33 days and complete return in from 3 to 8 weeks. It should be noted that these investigators attempted abdominal vagotomy, which in the dog can almost never be complete. When even a few fibers are left intact the effect of vagotomy may not be evident (7).

SUMMARY

1. Following partial gastrectomy and complete vagotomy there is profound decrease in acid secretion in response to a Liebig's extract test meal which is greater than after partial gastrectomy alone. In 51 experiments on four dogs, 17 or 33 per cent were characterized by anacidity.

2. Histamine caused a marked secretion of acid.

3. The intestinal phase of acid secretion was very marked and unusually prolonged.

4. Following operation the gastric samples contained large amounts of a very viscid mucus. When anacidity was present the amount of mucus was very great but it was usually not in evidence when large amounts of acid were being secreted in response to histamine and the intestinal phase. This suggests that the mucus is the result and not the cause of the lowered acidity.

5. The average acidity of the gastric contents of each of the four dogs was definitely below the threshold value of acid necessary for the digestion of living tissues as determined by Dragstedt.

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THE EFFECT OF ACETYLCHOLINE AND OTHER CONSTITUENTS OF THE SUPRARENAL GLAND UPON BLOOD SUGAR AND AMINO ACIDS

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The lowering of the amino acid content of blood in response to injected insulin (1) has been shown by Davis and Van Winkle (2) to be directly due to epinephrine secreted by the suprarenal medulla during insulin stimulation. The possibility that other constituents of the suprarenal gland might be involved in this phenomenon has now led us to investigate acetylcholine, ascorbic acid, and cortin—all of great physiological importance.

Of these substances it appeared most likely that acetylcholine would be active with respect to the blood amino acids. Since the work of Feldberg and co-workers (3) strongly suggests that epinephrine secretion results from a local action of acetylcholine, liberated at the nerve endings in the suprarenal medulla, it seemed possible that the injection of acetylcholine under appropriate conditions would bring about an epinephrine hypoaminoacidemia (cf. also Siehe, 4). Incidentally, the concentration of acetylcholine in the suprarenal medulla has been shown to be relatively high, as great as 0.45 mgm. per kgm. of medullary tissue; its concentration in the suprarenal cortex is much lower (5).

In addition to the amino nitrogen studies analysis of the blood with respect to reducing sugar was indicated, especially since the anticipated discharge of epinephrine might be of such a magnitude as to induce hyperglycemia. It should be noted that several investigators have studied the glycemic behavior of acetylcholine but have reported conflicting results. Bornstein and Vogel (6) and Seo (7) observed a hyperglycemia with acetylcholine, whereas Labbe *et al.* (8), Bruhn and Himwich (9), and Hrubetz (10) noted a diminution in blood-sugar content. Lang and Rigo (11) found that small doses produced hypoglycemia while larger doses were followed by hyperglycemia. The present investigation, we feel, throws further light on the glycemic behavior of acetylcholine and reveals the factors responsible for acetylcholine hyperglycemia.

We may state in advance that, according to our experience, acetylcholine tends to elevate the blood amino acids rather than to decrease

their concentration and that hyperglycemia from administered acetylcholine is only obtained if convulsions intervene. Neither hyperglycemia nor hyperaminoacidemia is dependent upon a discharge of epinephrine since acetylcholine produces a like response in the animal after destruction of the suprarenal medulla. Cortin and ascorbic acid were found to be inactive with respect to blood sugar and amino acids.

EXPERIMENTAL. *Animals.* Adult rabbits, chiefly female, weighing

TABLE 1

Tests of suprarenal medullary function in the operated animals with subcutaneous injection of insulin

ANIMAL NUMBER	WEIGHT	ABSOLUTE CONCENTRATION						PERCENTAGE VALUES					
		Blood sugar			Blood amino nitrogen			Blood sugar			Blood amino nitrogen		
		0 hr.	1½ hrs.	3 hrs.	0 hr.	1½ hrs.	3 hrs.	0 hr.	1½ hrs.	3 hrs.	0 hr.	1½ hrs.	3 hrs.
DOSE: 0.25 U./kgm.—Preliminary test													
	kgm.												
1	4.4				10.6	10.6	10.0				100	100	94
2	3.9				11.5	10.8	11				100	94	95
3	4.1				10.5	10	9.3				100	95	90X
4	2.5				13.1	12.3	13.0				100	94	100
5	2.2	130	40	C	9.1	9.5	8.9	100	31	C	100	104	98
6	1.9	113	40	C	10.2	9.9	9.6	100	35	C	100	97	84
7	2.2	130	66	C	9.2	9.1	8.5	100	51	C	100	98	92
8	2.7	108	51	C	9.5	9.8	9.5	100	47	C	100	103	100
9	4.0	112	68	102	11.1	10.3	9.5	100	61	91	100	92	86X
10	4.6	104	49	69	10.0	9.4	9.5	100	47	66	100	94	95
11	2.4	100	66	77	9.9	9.9	9.5	100	66	77	100	100	96
DOSE: 0.5 U./kgm.—Re-check on doubtful animals one month later													
3	4.1	99	37	28	10.1	9.7	8.7	100	38	28	100	96	86X
9	4.4	97	54	57	10.8	10.8	9.1	100	55	58	100	100	84X
7	3.6	90	52	57	10.7	10.8	10.5	100	58	64	100	101	98

C—Convulsion after 1½ hour sample—glucose administered and 3 hour sugar sample not significant.

X—Animal rejected.

from 2 to 4 kgm., were used. In a number of the animals the suprarenal medulla was destroyed by electric cautery. Histological studies on the suprarenals of such animals revealed an almost complete absence of suprarenal medullary tissue. The 11 animals demedullated by this procedure were subjected to functional tests to determine whether cauterization had been complete. Of the 9 animals retained none showed hyperglycemia after profound fright and none showed hypoaminoacidemia upon injec-

tion of insulin. The results of the insulin test, which we regard as conclusive (2), are presented in table 1.

Blood analysis. Samples of 2.5 cc. were removed from the marginal ear vein at intervals of 1, 2, 3 and 5 hours after commencement of an experiment. Pre-injection samples were also drawn for the determination of basal values. Larger blood samples were considered inadvisable because of the tendency of hemorrhage to induce hypoaminoacidemia (1, 12, 13, 14). The animals were fasted for 48 hours before use.

Blood sugar was determined by the micromethod of Folin (15) which proved to be advantageous because of the small sample (0.1 cc.) required for an analysis.

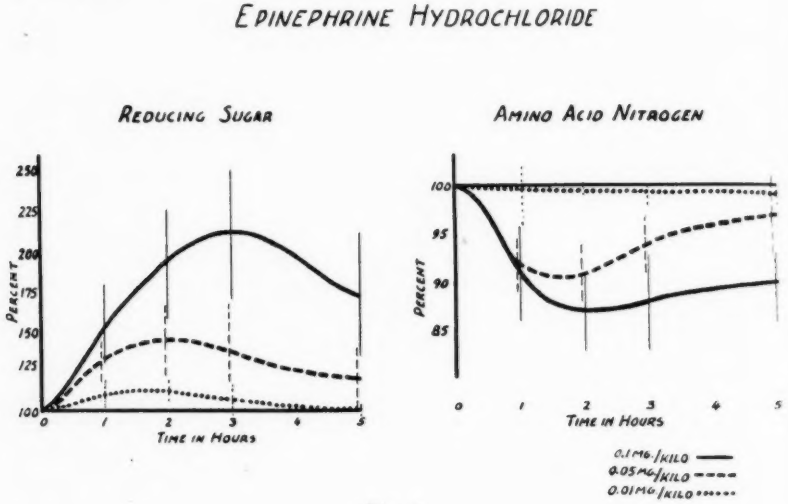

Fig. 1

Amino acid nitrogen was determined on 2 cc. portions by Danielson's modification (16) of Folin's method.

Administered substances. Epinephrine and cortical hormone preparations were obtained from Parke Davis & Co. to whom we are indebted for the gift of these substances. In addition to the crude preparations of the cortical hormone, a highly purified product, Eschatin no. 354, was also used.

Acetylcholine chloride (Hoffman-LaRoche), ascorbic acid (Merck), atropine sulphate (Merck), physostigmine sulphate (Merck), and insulin U 40 (Eli Lilly) were also used.

Experiments with epinephrine. The results of Luck and Morse (12) and Davis and Van Winkle (2) were confirmed, and a quantitative study

was made of the effects of various doses of epinphrine upon the blood sugar and amino acid nitrogen. The purpose of the experiments was to determine the minimum quantities of injected epinphrine necessary to elicit hyperglycemia and hypoaminoacidemia. The commercial preparation of epinephrine hydrochloride (1/1000) was diluted so that the drug was in such a concentration that one cubic centimeter of the solution was administered per kilogram of body weight. In this manner the same amount of diluting saline was administered in all cases, although the quantity of epinephrine varied. To three different groups, composed of at least six animals each, doses of 0.1, 0.05 and 0.01 mgm. of epinephrine per kilogram were injected subcutaneously.

The results of the experiments, recalculated to a common base line, and expressed in terms of percentage change from the pre-injection levels, are presented graphically in figure 1. Each point represents the average value for the group of six or more animals with the average deviation from the mean indicated by a vertical line passing through the point.

The absolute values obtained are indicated by the group averages and average deviations from the means presented in table 2.

The minimal hyperglycemic dose of epinephrine appears to be about 0.01 mgm. per kgm. body weight, a value which is evidently somewhat less than the minimal dose required to elicit hypoaminoacidemia.

In the procedures about to be described acetylcholine was administered in doses of 0.1 and 1.0 mgm. per kilogram of body weight both to normal rabbits and to rabbits in which the suprarenal medulla had been destroyed.

It has been shown by several investigators that acetylcholine is rapidly destroyed in the tissues by choline esterase. Loewi first noted this in his work on the "vagus substance" in 1921 (17). It was again reported by Loewi and Navratil (18), and Englehart and Loewi (19). For this reason acetylcholine must be administered intravenously. Subcutaneous, intramuscular, or intraperitoneal injections have no effect, and it is only by direct introduction into the blood stream, whence it can be rapidly disseminated, that it has any observable action. Even when injected intravenously its activity is of very short duration. Gaddum reports (20) that at 40°C. acetylcholine is almost completely destroyed by human blood in fifteen seconds. The procedure in these experiments was to inject small amounts into the marginal ear vein.

Acetylcholine rapidly deteriorates in solution. The chloride is a very hygroscopic substance, and therefore was kept in a desiccator over phosphorus pentoxide until the time for injections. As they were needed a few crystals were accurately weighed out, dissolved at a concentration of 1 mgm./cc. and injected. The animals convulsed if more than 0.1 mgm. was administered at any one moment. Two-tenths milligram, if given all at once, usually led to the death of the animal in violent convulsions

TABLE 2

Effect of suprarenal gland constituents on reducing sugar and amino acid nitrogen of blood

Group averages and average deviations from the means

SUBSTANCE ADMINISTERED	ANIMALS			REDUCING SUGAR						AMINO ACID NITROGEN					
				Hours after injection						Hours after injection					
	Dose, mgm. per kgm. body weight	Number in group	State	0	½	1	2	3	5	0	½	1	2	3	5
Epinephrine	0.1	6	Normal	117±19		179±12	221±21	243±38	198±25	8.4±0.4	7	7.7±0.5	7.4±0.4	7.5±0.5	7.7±0.5
Epinephrine	0.05	9	Normal	133±11		174±23	192±32	183±31	145±12	8.2±0.4		7.5±0.3	7.5±0.3	7.7±0.2	7.8±0.5
Epinephrine	0.01	6	Normal	108±24		117±22	126±26	116±23	117±18	8.3±0.4		8.2±0.1	8.3±0.4	8.1±0.5	8.2±0.4
Cortical hormone	0.5-1.0*	6	Normal	129± 7	130± 6	130± 6	125± 7	123± 4	122± 4	7.5±0.2		7.4±0.5	7.4±0.2	7.5±0.2	7.5±0.2
Eschatin	0.7*	6	Normal	122±11		122±14	125±12	124±10	128±13	8.8±0.8		8.6±0.8	9.2±0.6	8.9±0.6	8.4±0.6
Ascorbic acid	12-25	6	Normal	119± 5		117± 3	119± 4	120± 3	117± 4	9.8±0.9		9.8±0.7	10.1±0.7	9.8±0.7	9.5±0.7
Acetylcholine	1.0	10	Normal conv.†	125±11	190±21	169±29	161±29	139±25	129±13	8.5±0.4	11.2±0.8	10.5±1.2	9.1±0.7	8.6±0.7	8.7±0.9
Acetylcholine	1.0	9	Normal	121± 4		95±13	112± 8	123± 8	117± 7	9.7±0.7		10.6±1.1	9.8±1.0	9.4±0.9	9.4±0.7
Acetylcholine	1.0	8	S C†	119± 9		147±10	124±17	118±16	113±12	10.2±0.3		11.8±0.5	10.7±0.3	10.1±0.6	9.7±0.4
Acetylcholine	0.1	3	Normal	126± 6		121± 4	117± 3	121± 1	119± 0	9.2±0.6		9.5±0.3	9.5±0.3	9.4±0.2	9.1±0.2
Acetylcholine	0.1	3	N C†	103± 3		114± 7	109± 4	112± 3	109± 3	9.9±0.4		9.6±0.1	9.8±0.3	9.5±0.1	9.1± .0
Acetylcholine	0.1	7	S C†	120± 4		120± 6	117± 7	113±16	128±11	9.8±0.8		9.9±0.8	10.0±0.8	9.3±0.6	9.6±0.8
Atropine (2.0 mgm./kgm.) prior to acetylcholine (1.0 mgm./kgm.)		6	Normal	116±10	107±15	120±12	125± 4	132± 6	142±25	10.3±0.6	10.3±0.4	10.3±0.5	10.5±0.4	10.3±0.6	10.3± .5
Atropine	2.0	6	Normal	100± 4		103± 9	107±10	112±10	120±12	10.3±0.6		10.2±0.6	10.1±0.4	10.2±0.3	10.1±0.4
Physostigmine (0.2 mgm./kgm.) prior to acetylcholine (0.1 mgm./kgm.)		1	N C†	96	113	135	94	99	100	12.0	11.1	9.7	11.9	12.1	11.9
Physostigmine	0.2	3	Normal	116± 6		125±10	111± 3	115± 7	122± 5	10.2±0.7		9.9±0.6	10.0±0.8	10.1±0.6	9.9±0.6
				0	1	2	3	4	5	0	1	2	3	4	5
Insulin (1 unit/kgm.) prior to acetylcholine (1.0 mgm./kgm.)		3	Normal	132± 4	54	49± 3	75±13	85±24	86±16	11.4±0.4	10.7	9.8±0.2	10.3±0.8	10.0±0.4	10.4±0.4
Insulin (1 unit/kgm.) prior to acetylcholine (1.0 mgm./kgm.)		2	N C†	124± 1	50± 3	46± 1	118±31	131	139	10.2±0.4	9.4±0.2	8.3±0.1	9.7±0.5	8.7	9.5

* Cc. per kgm.

† N C—Normal, convulsed. S C—Suprareno-demedullated, convulsed.

which simulated the consequence of a profound cranio-sacral stimulation, showing a slow heart rate, profuse salivation and difficult respiration.

A quantity of 0.1 mgm. per rabbit was found to be a convulsive dose for half of the animals. This bore no relationship to the size of the animal, as most of the deaths were observed in those of greater weight. With a dose of 0.1 mgm. those that convulsed were only temporarily so affected. The effects of the drug wore off within a few seconds and after a minute or two another 0.1 mgm. could be given. In order to administer 1 mgm./kgm. it was necessary to give repeated injections of 0.1 mgm. each over the course of a half-hour. Such a dose was administered to a group of twenty rabbits. The time intervals for the subsequent blood samples

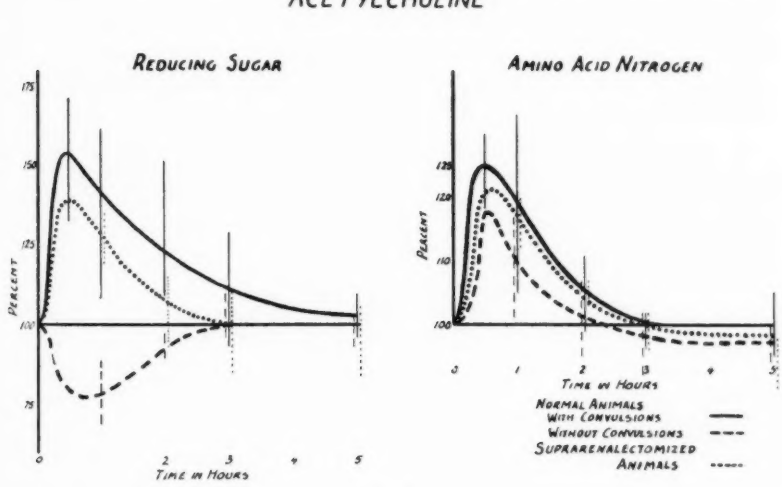

Fig. 2

were computed from the beginning of the injection period, and the thirty-minute sample was, therefore, frequently obtained within a few minutes of the completion of injection when this higher dosage was used. When 0.1 mgm./kgm. was administered there was necessarily a longer interval between the completion of the total dose and the taking of the first sample.

It was soon found that convulsions markedly altered the response to acetylcholine, and the data derived from these procedures have, therefore, been subdivided on this basis. It is interesting to note that animals which had very mild convulsions, sometimes only a few twitches or a single scream, exhibited the same biochemical responses as did those with more marked spasms, and clearly did not fall into the unconvulsed group.

The animals in which the suprarenal medulla had been destroyed were

more sensitive to acetylcholine than normals and convulsions intervened in almost all cases.

The results of the experiments are presented graphically in figure 2 in which group averages are plotted after recalculation to a common base line. Attention is drawn to the intimate relationship which exists between the convulsive state and hyperglycemia, to the hypoglycemia observed in the unconvulsed animals, to the hyperaminoacidemia which acetylcholine always induces and to the non-participation of the suprarenal medulla in respect to the changes both in blood sugar and amino acid nitrogen.

In table 2 the absolute values are expressed in terms of group averages and average deviations from the mean.

Several experiments were next undertaken in which acetylcholine was antagonized by the use of the parasympathetic inhibitor, atropine. Fifteen minutes prior to the use of acetylcholine the animals were injected subcutaneously with atropine sulphate, 2 mgm. per kgm. Such atropinized animals were completely resistant to acetylcholine irrespective of the rate of injection. Convulsions were never observed and, except for a delayed and mild hyperglycemia referable to the atropine alone, there was no effect upon either blood sugar or amino acid nitrogen.

These results are presented in table 2.

We next investigated the possibility of enhancing the action of acetylcholine by inhibition of choline esterase with physostigmine (18, 19). Three control experiments were performed with physostigmine alone. In three others, physostigmine sulphate, 0.2 mgm. per kgm., was injected prior to acetylcholine. It was possible to give only 0.1 mgm./kgm. of acetylcholine and, indeed, this dose induced such profound convulsions in the three rabbits that only one survived. This one animal demonstrated an hyperglycemia of 41 per cent over the preinjection value at the end of the 2-hour interval. The amino acid nitrogen meanwhile dropped to 80 per cent of the basal level. The sugars and amino acid level of the controls remained unaltered. Because of the high mortality accompanying this procedure further experiments were not undertaken, and since there is but one experiment no conclusions can be drawn.

Because of the fact that insulin reduces the amino acid nitrogen (1) and blood sugar of normal rabbits, a study was made of the effect of acetylcholine upon insulinized animals in which the blood had already undergone these changes. One unit of insulin per kilogram of body weight was given subcutaneously after the initial sample had been withdrawn. This was allowed to act for two hours until the insulin effect was at its maximum, and then 1.0 mgm. of acetylcholine per kilogram was given. Of the group of five animals so treated, two underwent convulsions. In these two the blood sugar and amino acid nitrogen rose rapidly.

Indeed, in one, the sugars rose from 38 per cent of the original value to 123 per cent in one hour and simultaneously the amino acid nitrogen rose from 84 per cent to 104 per cent of the preinjection level. The results are presented in figure 3. See also table 2.

The three animals that did not convulse showed no such effect. Again the convulsions appear to be of significance. It would seem as if the acetylcholine were acting here in the same manner as it did in the uninsulinized animals and that the effects of these two substances were quite independent.

Experiments with cortical hormone preparations. Two preparations of the cortical hormone were studied. The first was the commercial product

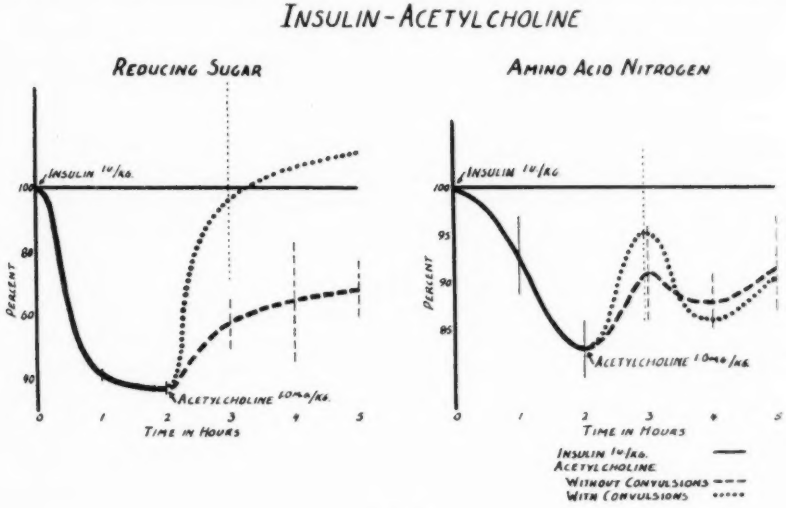

Fig. 3

Eschatin and the second was a more highly purified product known as Eschatin no. 354. These were injected subcutaneously into a group of rabbits in amounts ranging from 0.5 to 1.0 cc. of the extracts per kilogram of body weight. With these doses there were no alterations of the blood sugar or blood amino acid nitrogen levels. The old product Eschatin was administered to two different groups of three animals each, one of which received 0.5 cc./kgm. and the other 1.0 cc./kgm. The newer and more highly purified Eschatin no. 354 was given in a dose of 0.7 cc./kgm. to six rabbits. The data are presented in table 2. On reference to this, the absence of any changes in these blood constituents will be noted. We were quite unable to demonstrate any hyperglycemic effect with cortin, such as Collazo *et al.* (21) and Zunz and La Barre (22) reported; the

minor deviations which we observed all fall within the limits of experimental error.

Experiments with ascorbic acid (vitamin C). Two groups of three rabbits each were injected subcutaneously with 12 and 25 mgm. of ascorbic acid per kilogram of body weight. None of the animals underwent any change in the blood sugar or amino acid nitrogen content (table 2). The fact that these experiments were entirely negative, even with such large amounts of ascorbic acid would make it appear that this substance has no influence upon the blood sugar and amino nitrogen content of normal rabbits. Note that Stepp *et al.* report a marked hypoglycemia in humans after the intravenous injection of 300 mgm. of ascorbic acid (23).

Discussion. The results of most significance are those obtained from acetylcholine, which appears to exert a profound influence on the concentrations of blood sugar and amino acid nitrogen.

The mild hypoglycemia which acetylcholine induces in the unconvulsed animal can be explained most readily either by insulin secretion or inhibition of hepatic glycogenolysis. There is insufficient evidence as yet available to permit one to choose satisfactorily between these possible mechanisms. Experiments upon depancreatized animals are indicated. Hrubetz (10) has made similar observations in rats which gave a mild hypoglycemia in response to acetylcholine.

As for the hyperglycemia observed in certain of the acetylcholine experiments it is clear that epinephrine cannot be regarded as the causal agent. The work of Feldberg *et al.* (3) demonstrates that acetylcholine may be considered as the physiological agent responsible for the transmission of nerve impulses from the fibers of the splanchnic to the cells of the suprarenal medulla with resultant discharge of epinephrine. In our experiments, however, hyperglycemia was consistently observed after destruction of the suprarenal medulla and an epinephrine mechanism could not have been invoked.

We are more disposed to associate the hyperglycemia with convulsions, for elevation of the blood sugar was never observed in the unconvulsed animal but invariably followed upon convulsions, however brief. It seems most probable that the convulsive seizures initiate or accompany a chain of events in the liver which leads to hyperglycemia. The hepatic glycogenolysis, thus postulated, may be of an immediate nervous origin or the result of a humoral mechanism.

A second reason for excluding epinephrine as the hyperglycemic agent is based on the amino nitrogen determinations. It has been previously shown in this laboratory (2) that a discharge of epinephrine induces hypoaminoacidemia. Our present work indicates that the quantity of epinephrine required for this phenomenon is low,—the equivalent of that which would be absorbed from a subcutaneous pocket containing initially 0.01

to 0.05 mgm. of epinephrine per kilogram body weight. In the acetylcholine experiments, far from observing a hypoaminoacidemia, increases in the amino nitrogen content were actually obtained.

Such increases might also be interpreted as hepatic in origin, especially if any weight be given to the fact that hyperaminoacidemia is seldom observed in pathological states except as a consequence of certain severe hepatic lesions. Phenylhydrazine, probably acting as a liver poison, also induces hyperaminoacidemia (24). The immediate source of the blood amino acids, under physiological conditions, has never been revealed but it is not improbable that the liver is a major source.

Finally, we are tempted to suggest that the homeostatic mechanism responsible for regulation of the blood amino acid level may consist of epinephrine and acetylcholine. We have shown in the present research that acetylcholine induces hyperaminoacidemia while our earlier studies demonstrated the hypoaminoacidemic activity of epinephrine. It is also known that suprarenal infusions of acetylcholine provoke a discharge of epinephrine. To completely prove the reciprocal relationship which is now postulated it would be necessary to demonstrate that an over-discharge of epinephrine would elicit a secretion of acetylcholine and that the quantity of either substance in the general circulation or in certain tissues may be influenced by the amino acid level.

SUMMARY

1. The effect of epinephrine, acetylcholine, cortin, and ascorbic acid upon the blood sugar and amino acid nitrogen of rabbits has been studied.

2. Acetylcholine induces hyperglycemia in rabbits if convulsions intervene. In the absence of convulsions, acetylcholine causes a moderate degree of hypoglycemia.

3. Acetylcholine hyperglycemia is obtained in undiminished degree after destruction of the suprarenal medulla; the hyperglycemia is, therefore, not due to a discharge of epinephrine.

4. Acetylcholine causes hyperaminoacidemia both in normal rabbits and after destruction of the suprarenal medulla; it is independent of the incidence of convulsions.

5. Cortin and ascorbic acid are without effect on either blood sugar or amino acid nitrogen.

6. The minimum hyperglycemic dose of epinephrine is slightly smaller than the minimum dose requisite for the production of hypoaminoacidemia.

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A FURTHER STUDY OF THE RELATION OF THE ADRENAL CORTEX TO VITAMIN C[1]

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In an earlier report (Lockwood and Hartman) it was shown that adrenal cortical extract ameliorates the symptoms in avitaminosis C. The presence of vitamin C in the extract was ruled out by passing the preparation through ethyl ether in which it is insoluble. Grollman and Firor (1934) and Svirbely (1935) were unable to confirm these results. The present work confirms the earlier report and proves that the positive effects were not due to the presence of vitamin C. Three methods have been employed. First, the extract has been tested chemically for the presence of vitamin C. Second, the influence of graded doses of extract on the development of the avitaminosis has been determined because it is known that variations in the subminimal protective dose of vitamin C will affect the symptoms in proportion to the quantity given. Third, an extract of liver prepared by the same method as that used for cortical extract has been tried in avitaminosis C. This served as a check on the specificity of the action of the adrenal cortical extract. Liver is rich in vitamin C, although the content of the adrenals is always higher (Bessey and King, 1933; Svirbely, 1933; Yavorsky, Almaden and King, 1934). If the method used for the preparation of the cortical extract removes and preserves some of the vitamin from the adrenal tissue, the same should hold true for liver extract. The cortin content of the cortical extract was found by determining the minimum dosage required to maintain doubly adrenalectomized guinea pigs, of 300 grams weight, in good health. A control series was run in which twice the maximum amount (0.008 mgm. per cc.) of vitamin C, which could have been present in the extract, was injected daily into guinea pigs on a vitamin C-free diet.

The cortical extract was made by extracting the freshly ground adrenal cortex of beef with 95 per cent ethyl alcohol and passing successively through ethyl ether, 70 per cent alcohol (chilled), ethyl ether and crystallite. The finished extract was made up so that one cubic centimeter contained the product of 20 grams of fresh cortex and represented

[1] Aided by a grant from The Rockefeller Foundation.

the minimum daily maintenance dose of cortin for a 300 gram doubly adrenalectomized guinea pig.

Vitamin C determinations were made in a number of our extracts as a check on the method of preparation. In order to obtain maximum values for vitamin C the extract was treated with hydrogen sulfide, according to Tillmans, Hirsch and Dick. The results are given in table 1. Cortical extracts contain reducing substances other than vitamin C which accounts for the higher values given by the iodine method as compared with the indophenol (2,6-dichlorophenol indophenol) method. The extract used in this study was entirely from one lot (no. 131–4) which according to the iodine reduction method contained less than 0.008 mgm. of vitamin C per cubic centimeter. This amount is insignificant. The liver extract represented the product of 25 grams of fresh beef liver per cubic centimeter and contained 0.033 mgm. of vitamin C per cubic centimeter.

TABLE 1

Vitamin C content of extracts of adrenal cortex and of liver

EXTRACT NUMBER	DESCRIPTION	METHOD	VITAMIN C
			mgm. per cc.
136	Extracted with ether once	Indophenol	0.0282
139-41	Extracted with ether once	Indophenol	0.0080
160 S	Extracted with ether twice	Indophenol	0.0296
Ex 1	Purified	Indophenol	0.0034
Liver	Extracted with ether twice	Indophenol	0.0015
136	Extracted with ether once	Iodine	0.103
131-4	Extracted with ether twice	Iodine	0.008
Ex 1	Purified	Iodine	0.0528
Liver	Extracted with ether twice	Iodine	0.033

The spectrographic method was tried but proved less sensitive than the chemical methods for the determination of vitamin C because there was present in the extract an unknown substance whose absorption was near enough to interfere with that for vitamin C.

The experimental procedure followed closely that described by Sherman, LaMer and Campbell (1922). The guinea pigs were obtained from the same source and matched for weight and sex as in the earlier paper of Lockwood and Hartman. The experimental groups are as follows:

1. Positive control group receiving the basal vitamin C-free diet and 3 cc. of orange juice daily.

2. Cortical extract groups receiving the basal diet and two daily intraperitoneal injections of cortical extract (½ cc. each), a total of 1 cc. daily.

3. A group similar to 2, but receiving twice the amount of cortical extract, i.e., 2 cc. daily.

4. Another group similar to 2, except that the dose of cortical extract was re-

duced to the equivalent of 0.25 cc. daily, but given in a quantity of saline to equal in volume that of group 2, i.e., 1 cc. daily.

5. Liver extract group receiving the basal diet and the intraperitoneal injection of liver extract, ½ cc. twice daily; total 1 cc. per day.

6. Negative control group receiving the basal or vitamin C-free diet and daily intraperitoneal injection of 1 cc. of physiological salt solution.

7. Positive control group treated identically as group 1, but of lower average initial weight.

8. Vitamin C group, similar in weight to 7, receiving the basal diet and intraperitoneal injections of 0.016 mgm. of vitamin C (Merck) in physiological saline per day.

Groups 7 and 8 (broken lines, fig. 1) are not directly comparable with the other groups since the average initial weight of each is definitely

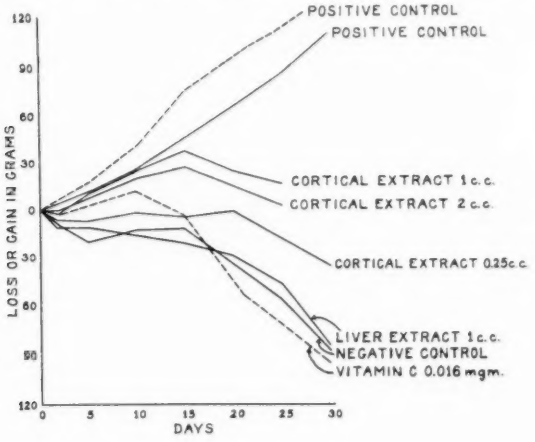

Fig. 1

lower, but they are comparable to each other and are the same as the above groups in all other respects.

Table 2 is a summary of the results obtained and figure 1 shows the average weight curves for each group.

As shown in figure 1, the maximum effect of cortical extract in retarding the development of vitamin C deficiency symptoms was obtained by the administration of 1 cc. It is interesting to note that this is the amount of extract that protected doubly adrenalectomized guinea pigs from developing symptoms of adrenal insufficiency. Two cubic centimeters of the same extract did not give greater protection. The lower average weight curve of these animals may be explained by the fact that the extract was slightly toxic and when administered in larger quantity proved irritating, with a tendency toward congestion at the site of the injection.

Even the 0.25 cc. daily injection of cortical extract gave a weight curve and scurvy score which indicated significant improvement over the negative controls. If the effects noted here, as the result of the use of adrenal cortical extract, were due to the presence of the vitamin, one would expect a graded response in protection in accordance with the quantity of extract given, since in every group receiving cortical extract the vitamin supply is less than a protective dose as the weight curves and scurvy scores indicate. The response is not in proportion to the extract used, which would seem to indicate that the active substance was not vitamin C. These results further show that a maximum influence of the cortical extract on the vitamin C deficiency condition is obtained with daily quantities of 1 cc. This maximum is far short of complete protection. If

TABLE 2

Influence of liver extract, vitamin C and graded doses of cortical extract on the development of scurvy

GROUP	NUMBER OF ANIMALS	EXPERIMENTAL PERIOD	AVERAGE INITIAL WEIGHT	AVERAGE FINAL WEIGHT	SCURVY SCORE
		days	*grams*	*grams*	
1. Positive controls	10	30	332	445	0
2. Cortical extract, 1 cc.	10	25	344	362	3
3. Cortical extract, 2 cc.	9	25	341	346	3
4. Cortical extract, ½ cc.	7	30	343	308	6
5. Liver extract, 1 cc.	9	30	332	268*	12
6. Negative controls	9	30	346	301†	17
7. Positive controls	8	28	310	441	0
8. Vitamin C (Merck), 0.016 mgm.	9	28	312	213‡	14

* Three animals dead.
† Four animals dead.
‡ Five animals dead.

vitamin C were the active constituent it should be possible to protect completely from avitaminosis C if a sufficient amount of extract were used.

As shown by the weight curve, the animals receiving liver extract are not measurably different from the negative control group. However, the scurvy score of the liver extract group is midway between the negative control and the 0.25 cc. cortical extract groups and is not far from that for 0.016 mgm. vitamin C. In each case the protective effect is so slight that it cannot definitely be interpreted as positive.

Discussion. Since the adrenal cortex is rich in vitamin C special precautions must be taken to avoid inclusion of the latter in an extract. This has been done in our work by two extractions with small volumes of ether at different stages in the process. The same method was used in the previous work. In order to make certain that this method leaves

the vitamin behind we have tested for its presence in two different ways, viz., by the iodine reduction method and by the indophenol reduction method and find that the amount present is insignificant. The same method of extraction applied to beef liver which, as already related, is rich in vitamin C (contains somewhat less than half the amount of that in the adrenal) (Svirbely, 1933) yielded an extract which contained no significant amount of the vitamin, as shown by the 2,6-dichlorophenol indophenol test and by animal assay. Therefore, the conclusion is justified that this method is capable of removing vitamin C and that the extract which we employed contained negligible quantities of this substance.

Dann and Cowgill (1935) have studied the vitamin C requirement of the guinea pig and concluded that 1 cc. of lemon juice per 100 grams of weight protects the animal from scurvy as determined by the Key and Elphick scale. Bezssonoff has found that 3 cc. of lemon juice daily is the minimum protective dose for a 400 gram animal. If lemon juice contains 0.6 to 0.7 mgm. of vitamin C per cc. (Harris and Ray, 1933) and we use Bezssonoff's value, then the daily protective dose of vitamin C for each guinea pig would be 1.8 to 2.1 mgm. Hou has found that injected vitamin C is twice as effective as that given by mouth. Therefore the daily protective dose by injection would be 0.9 to 1.05 mgm. The amount present in our extract is obviously insignificant.

Consideration of the question of the possible presence of vitamin C in the extract used in the first report is incomplete without comment on the work of Grollman and Firor (1934). They found that 0.25 mgm. of ascorbic acid intraperitoneally injected was definitely more effective in protecting an animal against scurvy, than the same quantity when fed. They concluded that "this is essentially the same result obtained by Lockwood and Hartman in their study of the comparative effects of the oral and intraperitoneal administration of cortical extract and we must conclude that their results are due to the relative efficacy of different modes of administration. Their extracts apparently contained about $\frac{1}{4}$ mgm. of ascorbic acid in 2 cc. (their dosage) which, as we have seen, is not unexpected." This interpretation of results based only on circumstantial evidence is not justified. Because they were unable to obtain positive results with their cortical extract they assume that our extracts must contain vitamin C. An essential difference between the extracts prepared in the two laboratories should be pointed out as it may have a significant bearing on the results. In the preparation of the extract of Grollman and Firor (1933), frozen adrenal tissue is extracted successively with acetone and benzene; the benzene solution is extracted with dilute alkali and then acid and the benzene removed *in vacuo* and finally the residue taken up in physiological saline.

We have found repeatedly that both acetone and benzene are poor

solvents for cortin. Moreover cortin is easily destroyed by dilute alkali. Although they injected the extract from 100 grams of adrenal tissue daily their only statement indicating its cortin content is "This amount of extract corresponds roughly to several times that elaborated by the adrenal glands of the normal guinea pig, as judged from its life-prolonging action in adrenalectomized animals." They give no actual assay value. It is important that the cat, guinea pig or dog be used for this purpose as the rat test is not so conclusive.

Svirbely has recently studied the effects of adrenal cortical extracts on avitaminosis C. He found that ethyl ether does not dissolve the vitamin in significant amounts. The extract which he used was furnished by Dr. E. C. Kendall. Although assayed, its potency is merely stated, "The amount injected in the normal guinea pig should have been adequate (based on assay) to prevent suprarenal deficiency in adrenalectomized animals." He used only 0.2 cc. per day per animal. The dose may have been too small to produce the marked effects which we obtained.

The present work confirms our earlier report that either cortin or some unknown substance from the adrenal cortex delays the onset of scurvy.

SUMMARY AND CONCLUSION

An extract of the adrenal cortex relatively free from vitamin C has been prepared. This extract ameliorates the symptoms of avitaminosis C. An extract of the liver prepared in the same way offers no protection in this avitaminosis. There is an amount of cortical extract which furnishes optimum protection. Greater amounts fail to increase it although the protection is far from complete. Therefore the substance cannot be vitamin C. Cortin or some unknown substance from the adrenal cortex delays the onset of scurvy.

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THE EFFECT OF OCCLUSION OF THE OUTFLOW OF PROSTATIC SECRETION ON THE PROSTATE GLAND

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The effect of occlusion of the outflow of prostatic secretion on the prostate gland has not been studied as far as we have been able to ascertain. The occlusion of the ducts of other external secreting glands such as the salivary glands, the pancreas and liver, as is well known, causes degenerative or atrophic changes in the external secreting cells. Although we anticipated a similar change in the case of the prostate, we thought that hypertrophy might conceivably result. One could not even predict that atrophy would result, because the continuous secretion of the prostate is relatively small. Further, we were interested in ascertaining whether corpora amylacea might be produced under conditions of obstruction of the outflow of prostatic secretion.

METHODS. Three male dogs about four years old were operated on by the following procedure: an incision was made suprapubically to expose the bladder neck and prostate gland. The posterior urethra was grasped between clamps and divided. The cut ends were closed by a catgut suture. Since the prostate in the dog can easily be separated from the vesical neck, this was done, and the cut edge of the bladder closed, and the cut end of the urethra sutured. Care was taken to interfere as little as possible with the blood supply of the gland. A suprapubic cystotomy was done to allow the urine to drain externally (fig. 1).

The circumference of the gland in each dog was measured by placing a string around the gland at its greatest circumference. The dogs were then allowed to survive for a period of five months.

The glands were again measured at autopsy. In addition, histological sections were made of the three glands, and also of normal glands for comparison.

RESULTS. That the operation per se does not lead to significant atrophy was observed in a previous study (1) by making a fistula of the urethra through which the prostatic secretion could drain. In such animals the prostate continues to secrete indefinitely, i.e., at least for seven months.

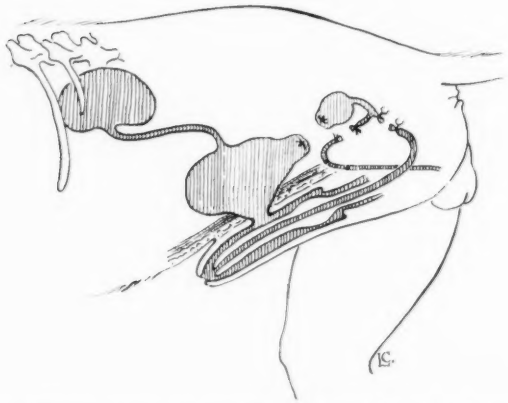

Fig. 1. This diagram shows the method employed for obstructing the secretion of the prostate.

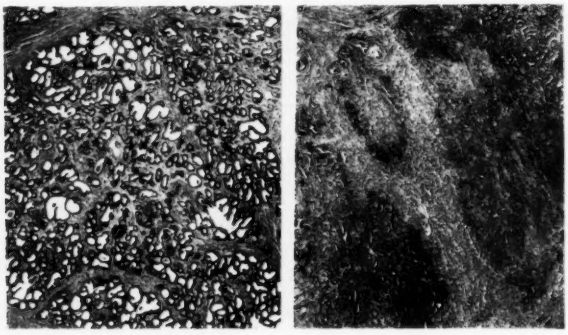

Fig. 2. The section on the left is from a normal prostate; that on the right is from the obstructed prostate of dog 2. The atrophy of the secretory cells is very evident.

TABLE 1

Changes in the circumference of the prostate

DOG	BEFORE	AFTER
1	$4\frac{1}{2}$ inches (11.5 cm.)	3 inches (7.6 cm.)
2	5 inches (12.8 cm.)	$2\frac{7}{8}$ inches (6.9 cm.)
3	5.2 inches (13.1 cm.)	3 inches (7.6 cm.)

In each of the three dogs after five months of obstruction, the external secreting cells were found to be degenerated (see fig. 2), but not completely; an apparent or a relative (not a real) increase in gland stroma occurred. The greatest external circumference of the gland decreased (table 1).

CONCLUSIONS

Obstruction to the outflow of secretion from the prostate gland by means of ligating the urethra causes an atrophy of the external secretory cells. Neither hypertrophy nor corpora amylacea were observed. The prostate gland reacts to obstruction to the outflow of its external secretion as do other externally secreting glands.

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THE RÔLE OF THE ANTERIOR HYPOTHALAMUS IN TEMPERATURE REGULATION

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It is now known that the hypothalamus serves as a thermostat regulating body temperature (Isenschmid and Schnitzler, 1914; Keller and Hare, 1932a, b; Bazett, Alpers and Erb, 1933; Ranson and Ingram, 1935; Davison and Selby, 1935). It remains, however, to determine more accurately what part of the hypothalamus serves this function and whether or not one and the same hypothalamic mechanism protects the animal against both rises and falls in external temperature.

METHODS. Adult cats and some that were about two-thirds grown, in good health and free from infections were selected. Lesions were placed with the Horsley-Clarke apparatus in the anterior part of the hypothalamus by Dr. W. R. Ingram for the purpose of producing diabetes insipidus as in earlier experiments reported by Fisher, Ingram and Ranson (1935).

Records of rectal and room temperatures were made daily after the operation for two weeks or until the temperature, if abnormal, had become normal again. Observations were made and duly recorded as to the condition of the cat and whether or not there was any evidence of infection.

In order to determine the ability of the animals to regulate against changes in environmental temperature, cold box and hot box tests were devised. Both normal and operated cats were always tested under the same conditions.

The temperature in the cold box ranged from 30 to 42°F. and the cats as a rule remained in the box for three hours. The hot box (30 x 24 x 12 inches) was regulated as nearly as possible at 102 to 104°F. and was equipped with wet and dry bulbs for humidity determinations. A small stream of air accurately controlled as to volume was passed through the box to keep the humidity from being raised too high by evaporation from the animal's lungs. For the tests in the hot box, the animals were restrained in a comfortable hammock made of canvas stretched on a frame that raised the hammock five inches above the floor of the box. The cats were kept under observation for many weeks and were finally sacri-

ficed to determine the location of the lesion by microscopic examination of the brain.

Rectal temperature readings on the days immediately following the operation. The cats were kept in an incubator regulated at 84 to 86° during the first day or two and sometimes longer as indicated by the stars following the rectal readings in table 1. Even under these conditions five showed slightly subnormal temperatures on the morning after the operation. Each of these five was somnolent the first morning due to prolonged action of the nembutal or to the effect of the lesion in the hypothalamus.

TABLE 1

Morning rectal temperature (F.) on the first eight postoperative days

CAT NUMBER	1	2	3	4	5	6	7	8
20	104.5*	105.6	104.6	103.7	103.2		102.0	102.8
21	97.0*	99.3*	101.3*	98.8		99.3	99.6	100.2
22	101.2*†	103.6*	102.5*	102.7	101.5	102.7	101.0	102.8
23	103.0*†	105.0*	103.2	102.6	101.5	101.2	101.8	100.4
24	102.2*	102.2*	103.2	102.9	101.3	99.6	99.3	100.0
26	87.8*†	100.5*	101.1*	104.6*	101.0*	102.1*	103.6*	102.8*
27	104.4*	103.4*	103.1	104.2	101.5	100.5		101.2
28	104.0*	105.1*	104.3	103.2	103.2	101.5	102.6	101.6
29	94.8*†	104.5*	102.1	101.7	102.3	101.0	98.7	100.1
30	103.9*†	104.2*	103.7*	103.3	105.2	102.6	103.9	103.2
31	102.8*	104.3*	104.0*	103.7*	101.6*	103.4*	101.6*	
32	102.6*	102.8*	102.7*	102.4*			102.0*	101.8*
33	95.0*†	95.3*	102.3*	103.7*	104.1*	104.2*	104.3*	102.9*
34	103.9*	99.9*	100.0*	98.0	97.9	96.8	98.3	99.0
35	105.0*	104.6*	103.5*	105.6	104.7	102.6	103.7	100.8
36	106.1*	103.7*	104.3*	104.7*		107.1*	105.7	103.8
37		104.5*	105.2*	101.7	100.5	100.6	101.7	100.8
38	99.3*†	104.1*	103.5*	103.4*	102.0	101.2	101.9	101.1

* Indicates cat was in the incubator (84–86°F.).

† Indicates cat was still somnolent when temperature was taken.

Cat 33 had an infected ear and cat 38 a respiratory infection.

None was somnolent on the second postoperative day. On the third, fourth or fifth day most of the animals were removed from the incubator without developing subnormal temperatures. Only one cat, 34, ran a temperature much below 99°. Cats and monkeys in which lesions had been placed in the posterior part of the hypothalamus have in our experience shown very much greater loss in the capacity to maintain body temperature up to the normal level.

If 101° or 102° be taken as the normal rectal temperature for the cat it will be seen that a number of the operated animals ran temperatures

distinctly above normal in the incubator and in some cases several days after the operation when they were being kept under ordinary room conditions. It would appear that insofar as these results show a disturbance in temperature regulation there was more often a loss of capacity to keep the temperature down to normal than up to normal. One of the animals (33) had an ear infection and another (38) had snuffles but none of the others had any infection to explain the high temperature.

Results of tests in the cold box. From observations on twenty-nine nor-

TABLE 2

Tests in hot box—Normal cats

CAT NUMBER	TEMPERATURE WHEN PANTING BEGAN	RISE BEFORE PANTING BEGAN	TIME BEFORE PANTING BEGAN
	°F.	°F.	*minutes*
1	103.7	2.1	32
2	103.6	0.9	20
3	104.2	2.0	40
4	103.2	0.5	30
5	103.4	1.0	30
6	102.0	0.4	8
7	103.7	1.1	20
8	104.3	1.3	27
9	104.6	0.8	11
10	102.5	0.7	14
11	104.2	1.7	53
12	103.0	1.2	29
13	102.3	0.8	12
14	102.8	0.5	27
15	102.4	0.8	10
16	102.9	1.8	50
17	103.4	2.4	45
18	104.0	1.9	93
19	103.6	2.6	148
20	103.7	2.6	30
Average.....	103.4	1.4	36

mal cats, including one castrate, subjected to cold air ranging from 30 to 42° for a period of three hours, it was seen that the rectal temperature rose slightly, remained the same, or fell slightly. The highest rise observed was 1.7° and the greatest fall was 0.9°. In no case was the temperature at the end of the period below 100°.

Of thirty-seven operated cats tested as to their ability to maintain normal temperatures against exposure to cold, twenty-six maintained their temperature within the normal range, eleven showed deviations from the normal. In these eleven the rectal temperature in four fell to a

point between 99° and 100°, in three others to a point between 98° and 99° and in only four did it fall below 98°, reaching 96.9°, 96.3°, 95.6° and 94.0° respectively. Shivering was seen in these operated animals as regularly as in the normal controls. It developed in those whose temperature fell below normal as well as in those in which no significant fall occurred. These cold box tests were made several days or weeks after the operation and it is possible that had the tests been made one or two days following the operation more severe temperature disturbances would have been noted.

Results of tests in the hot box. The experiments with normal cats showed a great deal of variation among different cats and in different trials on the same cat as to rise of rectal temperature before panting set in and the time required for this response.

The response of normal cats was as follows: Immediately after the cat was put into the box, the rectal temperature began to rise (unless it was already over 102.0° as a result of struggling in the hammock or excitement, in which case it sometimes fell slightly, and a little later began to rise). With the rise in temperature there was always a concomitant increase in the rate of respiration which rose rapidly and steadily until panting appeared, then the rate of panting continued to increase at intervals with the further rise in temperature until a rate of 200 to 300 per minute was obtained with a temperature of 105.0°. The appearance of panting showed marked variations (table 2); the latent period varied from 8 minutes to 148 minutes; the rise in rectal temperature before panting began varied from 0.4° to 2.6°, with an average of 1.4°F.; the temperature at which panting began varied from 102.0° to 104.6° with the average point at 103.4°. It is to be noted that normal cats do not maintain normal temperatures under these external conditions, but physiological mechanisms are invariably called into play to their fullest extent: polypnea, cutaneous vaso-dilatation as seen on the ears and pads of the feet, and sweating on the feet. Salivation was noted along with the polypnea. As the rectal temperature rose the cats became more and more excited; frequent cries, dilated pupils, and struggling were observed with the animals obviously in distress.

The operated cats presented responses to heating which in most cases were abnormal. Panting began at a higher rectal temperature than in normal cats. Many did not pant although their rectal temperature reached the high levels of 105° or 106° (table 3). Some did not even exhibit an increase in respiratory rate at all at these temperatures; in most cases there was no sweating on the pads of the feet; a few of the animals showed a surprising tolerance to the heat and an apparent indifference to the hyperpyrexia, lying still and resting with normal respiratory rate, seemingly very calm and in no distress whatsoever.

The gradient of rise in rectal temperature when this is plotted against the respiratory rate was much steeper in the operated than in the normal animals (fig. 1). The steepness of this gradient is as significant as the

TABLE 3

Tests in hot box—Cats with anterior hypothalamic lesions

CAT NUMBER	DAYS AFTER OPERATION	TEMPERATURE WHEN PANTING BEGAN	RISE BEFORE PANTING BEGAN	TIME BEFORE PANTING BEGAN	TIME IN BOX	TEMPERATURE REACHED WITHOUT PANTING
		°F.	°F.	*minutes*		°F.
1	36	107.0	4.2	93		
2	36	105.0	4.5	180		
3	29	104.7	4.8	160		
4	27	106.4	6.5	120		
5	28	103.8	2.8	110		
6	32	103.2	2.8	100		
7	28	105.6	4.0	72		
8	20	104.1	2.1	100		
9	20	105.0	3.2	51		
10	20	105.0	3.2	60		
11	33	104.9	3.4	80		
12	20				190	106.4
13	12				210	105.8
14	11				140	106.3
15	10	104.4	4.4	140		
16	9				148	106.2
17	7				26	106.4
18	32	103.4	3.4	84		
19	130	105.3	7.3	238		
20	17	105.3	3.5	122		
21	17	104.3	1.6	64		
22	24	104.5	3.3	134		
23	21	105.9	4.4	100		
25	27	106.0	4.6	127		
26	27				170	103.3
27	14	105.3	4.7	86		
28	11				47	106.3
29	9				102	106.2
31	91				180	104.8
32	29				170	106.3
33	38	105.6	4.2		120	
38	16	105.0	3.5	250		
Average.....		105.0	3.9	117.7	136.6	105.8

temperature at which panting occurred. Thus, cats 5 and 6 which panted at temperatures below 104° gave data which when plotted showed steep gradients similar to those of the other operated cats. Three cats (8, 18

and 21) gave data which when plotted yielded curves more like the normal and these cats also panted at moderately low temperatures. These three cats, therefore, gave fairly normal reactions in the hot box.

It is clear that the capacity to regulate against heat may be greatly impaired without any loss in the ability to regulate against cold. For instance, four cats in which the hot and cold box tests were both made within a few days after the operation reacted normally against cold but failed to pant even when the rectal temperature was raised above 106° (cat 14, cold box test on 9th and hot box test on the 11th day; cat 16, cold box test on the 7th and hot box test on the 9th day; cat 17, cold

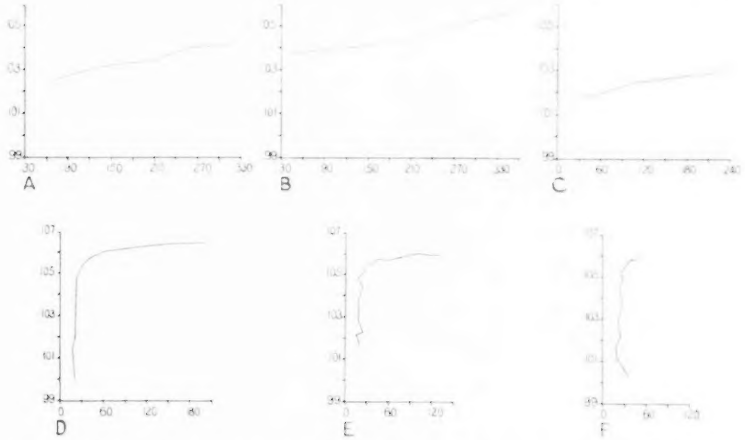

Fig. 1. Graphs showing rise in rectal temperature in degrees F. plotted against the number of respirations per minute and illustrating the effect of heating the animals in a box maintained between 102 and 104 F. A, B and C, three normal cats; D, E and F, three cats with lesions in the anterior hypothalamus; D, cat 4; E, cat 25; F, cat 16.

box test on the 6th and hot box test on the 7th day; cat 28, cold box test on the 9th and hot box test on the 11th day).

As a result of the much greater respiratory rate, the normal cats eliminated more water from their lungs and caused a more rapid rise in the humidity of the air in the box. The average increase in humidity divided by the average number of minutes that the animals were in the box gives a humidity quotient of 0.1275 for the normal cats and 0.057 for the operated series. In other words, the greater increase in respiration in the normal cats resulted in more than twice as rapid an increase in the humidity of the air in the box as occurred when the operated animals were being tested.

Cat 27 may be taken as typical of those which showed a decreased re-

sistance to heat. This animal ran a temperature definitely above normal, varying between 103.1° and 104.4° during the first four postoperative days (table 1). During the first two days it was in the incubator; but normal cats maintain normal temperatures under these conditions. During the third and fourth days the cat was in a room the temperature of which varied between 68° and 70°F. Twelve days after the operation the cat was placed in a cold box (30 to 42°) for two hours and fifty-five minutes and its rectal temperature dropped from 101.3° to 100.9° or only 0.4°F. On the fourteenth postoperative day it was put in the hot box and its temperature reached 105.3° before panting began. The curve of temperature plotted against respiratory rate had a steep gradient.

The lesions in this cat damaged extensively the optic chiasma and the

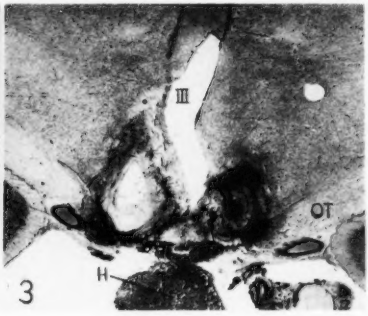

Fig. 2 Fig. 3

Fig. 2. Section through the hypothalamus of cat 27 showing involvement of the optic chiasma and the suprachiasmatic region. F, fornix; III, third ventricle. Weil stain.

Fig. 3. Section through the hypothalamus of cat 27 showing destruction of the infundibulum and adjacent part of the floor and walls of the third ventricle. *H*, anterior lobe of hypophysis; *OT*, optic tract; *III*, third ventricle. Cresyl violet.

supraoptic portion of the hypothalamus (fig. 2) extending backward through the tuber destroying the infundibulum (fig. 3). The lesions on the left side extended backward almost to the mammillary body but that on the right left much of the ventromedial hypothalamic nucleus intact. The lesions are somewhat larger and extend further dorsally and laterally than in most of the cats of this series. Extensive damage was done to the optic chiasma, supraoptic commissures, anterior hypothalamic area and nucleus and to the dorsomedial and ventromedial hypothalamic nuclei. Some damage was done to the filiform and supraoptic nuclei and the nucleus ovoideus was destroyed on one side.

In all of the animals studied in this investigation the lesions involved the anterior part of the hypothalamus including the floor of the third

ventricle as far back as the infundibulum. In most instances more or less extensive damage was done to the optic chiasma. Exceptions to this rule are furnished by two of the three cats which showed fairly normal reactions in the hot box. In cat 8 the lesion reached forward as far as the posterior border of the optic chiasma on one side only and in cat 21 the lesions were in the tuber and did not even approach the chiasma. The brain of the other cat which behaved normally in the hot box is not available for study.

In those cats which were unable to keep the rectal temperature quite up to normal during exposure to cold the lesions extended farther backward than usual destroying the rostral part of the posterior hypothalamic nucleus and in a few cases also the rostral part of the mammillary bodies. These observations on the cat are in agreement with the results of experiments made on the monkey which have shown that a transient hyperthermia may result from lesions confined to the anterior part of the hypothalamus while bilateral lesions which extend back dorsolateral to the mammillary bodies cause hypothermia (Ranson, Fisher and Ingram, 1937).

The data available are not sufficient to enable one to determine just what structure in the anterior part of the hypothalamus is concerned in holding the body temperature down to normal under conditions which tend to cause a rise. But it seems clear that a mechanism responsible for such regulation exists in this region. Of equal interest is the fact that such extensive damage to the anterior part of the hypothalamus involving the suprachiasmatic region and the floor of the third ventricle back to and including the infundibulum causes no obvious disturbance in the capacity to prevent abnormal falls in body temperature. These results are not in agreement with those of Frazier, Alpers and Lewy (1936).

SUMMARY

Cats with lesions in the anterior part of the hypothalamus ran normal temperatures or in some cases even showed a slight hyperthermia. They showed a diminished capacity to prevent abnormal rises in body temperature and when placed in a box at 102 to 104° they did not react as readily as normal cats by increased respiratory rate and panting. The ability to regulate against cold was not seriously impaired in these animals.

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EMOTIONAL LEUCOPENIA IN RABBITS[1]

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Several recent investigators have shown that emotional states influence the total number of white blood cells in the general circulation. Mora, Amtman and Hoffman (1926) found an emotional leucocytosis ranging from 30 to 150 per cent in dogs and cats. Menkin (1928) observed an emotional mononucleosis in cats. Cheng (1930) and Nye and Barrs (1932) reported that restraint of rabbits for a few hours in the abdomen-up position caused a leucopenia. Cheng (1930) reported this to be a lymphopenia. On the other hand Schweizer (1932) found no apparent relation between the position of a rabbit and the leucocyte count, and Katsura (1930) described a leucocytosis in the blood of rabbits one hour after binding.

In this study we have investigated the number of leucocytes in the blood during emotional states.

PROCEDURE. Rabbits were chiefly used in our experiments, but for comparative purposes a group of cats were also employed. All of our animals were full grown and both sexes were represented.

The blood was obtained from a needle puncture in a marginal ear vessel in the rabbits and from the cats by snipping the margin of an ear with a sharp pair of scissors for each sample. The first drops of blood were always discarded. The ear vessels were slightly dilated by warmth from an electric bulb before securing blood.

Central blood from the rabbits was taken directly from the heart by means of a hypodermic syringe.

All animals were deprived of food and water for 16 to 24 hours previous to an experiment so as to minimize the effects of digestion and absorption on the blood picture.

For obtaining blood for all the counts on peripheral blood in the normal and recovery periods the rabbits and cats were in the quiet upright position. But during excitement they were restrained for about 10 minutes back downwards to an animal holder and excited by being teased with a

[1] A preliminary report was presented to the American Physiological Society March 1934.

weak interrupted faradic current for approximately 3 minutes. All of our animals showed distinct signs of sympathetic stimulation under excitement.

The blood was collected in standardized Trenner automatic pipettes and each sample diluted with a one per cent acetic acid solution which was slightly colored with gentian violet. A uniform procedure was followed in diluting the blood, shaking the pipettes, filling the hemocytometers and

TABLE 1

Protocols. The number of leucocytes in the peripheral blood before, during, and after emotional excitement

SEX	FIRST NORMAL	SECOND NORMAL	EXCITED	FIRST RECOVERY	SECOND RECOVERY
			A. Normal rabbits		
F	15,300	15,450	10,200	13,500	15,100
F	17,700	14,500	13,100	18,500	20,000
M	9,800	7,800	6,900	6,200	6,200
M	10,100	11,400	8,300	9,000	8,750
F	8,400	7,600	7,300	7,200	9,500
Average....	12,260	11,350	9,560	10,880	11,910
			B. Splenectomized rabbits		
F	11,500	15,500	5,200	12,700	15,000
F	33,200	28,900	12,200	29,500	25,700
M	8,600	9,350	6,200	8,750	9,000
M	14,970	12,500	10,600	14,500	13,000
F	6,600	8,600	4,950	5,950	6,100
Average....	14,974	14,970	9,830	14,280	13,760
			C. Normal cats		
F	26,100	28,300	47,000	37,700	34,200
F	18,000	18,100	26,500	21,400	22,000
M	11,100	11,550	14,750	13,900	12,900
M	14,500	14,500	24,000	14,300	14,500
Average....	17,425	18,113	28,063	21,825	20,900

in the counting of the cells in every case. At least 400 cells were enumerated in each test. The values recorded are the averages of the counts from two double chambered Neubauer ruled hemocytometers which were filled from the same diluting pipette.

RESULTS. *Peripheral blood.* A series of five counts at 15 minute intervals, two in the normal state, one in the excited state and two during recovery were made on the peripheral blood in a group of 23 tests on rab-

bits. The average of the counts in the normal quiet state was 10000 per cu. mm. of blood, during excitement 8300, and during recovery 9300. The average decrease during excitement was 17 per cent. In table 1-A protocols from this group of animals are shown.

In a second series of 14 normal rabbits four consecutive counts at 15 minute intervals were made in the normal quiet state, one during excitement and four during recovery. These counts as in the previous group varied in the different individuals, but on the whole they showed a decided leucopenia during excitement. The average of the counts in the quiet state was 11000 and during excitement 7490, a decrease of 31.9 per cent below the normal level. The four recovery counts averaged 10150.

Central blood. In order to compare the effect of excitement on the

TABLE 2

Average changes in the number of leucocytes in the peripheral blood of normal rabbits and cats, the heart blood of normal rabbits and the peripheral blood of splenectomized rabbits

TYPE OF ANIMAL	NUMBER OF TESTS	TOTAL LEUCOCYTES—QUIET STATE	TOTAL LEUCOCYTES DURING EXCITEMENT	PER CENT CHANGE
Normal rabbits (P)	23	10,000	8,300	−17.0
Normal rabbits (P)	14	11,000	7,490	−31.9
Normal rabbits (H)	19	9,000	7,800*	−14.3
Splenectomized rabbits (P)	17	14,100	7,500	−45.4
Cats (P)	10	16,100	22,800	+41.6

P = Peripheral blood; H = Heart blood.

* Delayed leucopenia.

number of leucocytes in central blood with that in peripheral blood a series of 20 counts was made on blood obtained from the hearts of rabbits. These averaged 9000 in the quiet state, 9100 immediately after excitement, 7,800 during the first recovery and 8700 during the second recovery. Thus there was no immediate leucopenia, but a delayed one of 14.3 per cent.

Effect of splenectomy. In a series of 16 tests made on splenectomized rabbits the number of white cells in peripheral blood in the normal quiet state averaged 14100, during excitement 7500, while during recovery it was 14035 or back to the normal condition for this group. In these experiments there was a decrease of 45.4 per cent in the number of leucocytes during excitement. The removal of the spleen seems to have intensified the leucopenia. Table 1-B gives protocols from this group.

Cats. In order to find whether the leucopenia observed in rabbits

occurs also in cats under a similar condition of emotional excitement, a series of ten observations was made on the peripheral blood of these animals in the normal, excited, and recovery states. In contrast to the leucopenia found in rabbits each one of these cats showed a decided leucocytosis during emotional excitement. The number of leucocytes averaged 16,100 in the normal quiet state and 22800 during excitement, which is an increase of 41.6 per cent, while during recovery the counts averaged 17125. Protocols are shown in table 1-C.

The five groups. Table 2 gives a summary of the average changes in the number of leucocytes in each group of our animals during excitement. Although there was a decided leucopenia in the peripheral blood of all of our rabbits and a marked leucocytosis in cats under emotional excitement, there was a decided decrease in the number of polymorphonuclear leucocytes with a concomitant increase in the lymphocytes in all of these groups of animals.

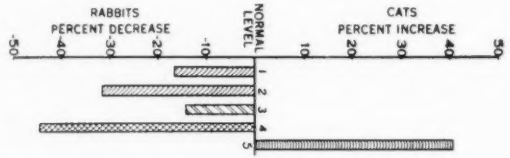

Fig. 1. Summary of percentage changes in the number of leucocytes.
1 = 23 tests on peripheral blood of normal rabbits. *2* = 14 tests on peripheral blood of normal rabbits. *3* = 19 tests on heart blood of normal rabbits. *4* = 17 tests on peripheral blood of splenectomized rabbits. *5* = 10 tests on peripheral blood of normal cats.

Figure 1 shows the average percentage changes in the number of leucocytes from the normal level in each one of our groups of rabbits and cats during emotional excitement.

Discussion. Our average normal leucocyte values of 9000 to 11000 per cu. mm. of blood are within the range reported for rabbits by Pearce and Casey (1930) and Jackson and Stovall (1930). In this investigation we have been concerned with relative changes in the number of white blood cells during quiet and emotional states and not with absolute values.

A considerable amount of evidence indicates that leucocytes may be sequestered in the capillaries or be mobilized into the blood stream during emotional states. (For a review see Garrey and Bryan, 1935.) Wells (1917) reported an increased number of neutrophiles in the capillaries of the liver, lungs and spleen during leucopenia caused by injecting dead bacteria into rabbits. Shigeru Momouye (1929) showed histologically that large numbers of leucocytes are immobilized in the capillaries of the lungs, intestine and bone marrow. Nye and Barrs (1932) reported an

abundance of granular leucocytes present in the liver, lungs and to a less extent in the spleen. Seyderhelm and Oestrich (1927) described the filtering and sequestering action of the lungs, liver, and spleen.

In the emotional leucopenia in our rabbits and in the leucocytosis in the cats there was a decided decrease in the percentage of the polymorphonuclear leucocytes in the blood with a concomitant increase in the lymphocytes. This will be described in a forthcoming communication.

Our results with the leucopenia in rabbits agree in part with Cheng (1930) and Nye and Barrs (1932). The leucocytosis found in cats is in agreement with other workers.

SUMMARY

This investigation was undertaken to determine the effect of emotional excitement on the distribution of leucocytes in the blood of rabbits.

In a series of 23 tests on the blood of rabbits in the normal quiet state the total number of white blood cells in peripheral blood averaged 10000 per cu. mm. of blood. During excitement this number decreased to 8300 or 17 per cent, showing a definite leucopenia.

In a series of 14 tests on the peripheral blood of a second group of normal rabbits the average white cell count was 11000 per cu. mm. of blood and during excitement 7490, which is a reduction of 31.9 per cent.

A series of 20 tests on blood taken directly from the heart showed a delayed leucopenia of 14.3 per cent after excitement.

In a series of 16 tests on splenectomized rabbits the number of leucocytes decreased from 14100 in the normal quiet state to 7500, or 45.4 per cent during excitement.

There was a decided leucocytosis during excitement in cats. The total number of leucocytes in the peripheral blood in a series of 10 consecutive tests on cats averaged 16100 per cu. mm. of blood in the quiet state and was augmented to 22800 or 41.6 per cent during excitement.

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